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PLANT AND SOIL

International Journal of Plant Nutrition, Plant Chemistry, Soil Microbiology and Soil-borne Plant Diseases

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PLANT AND SOIL

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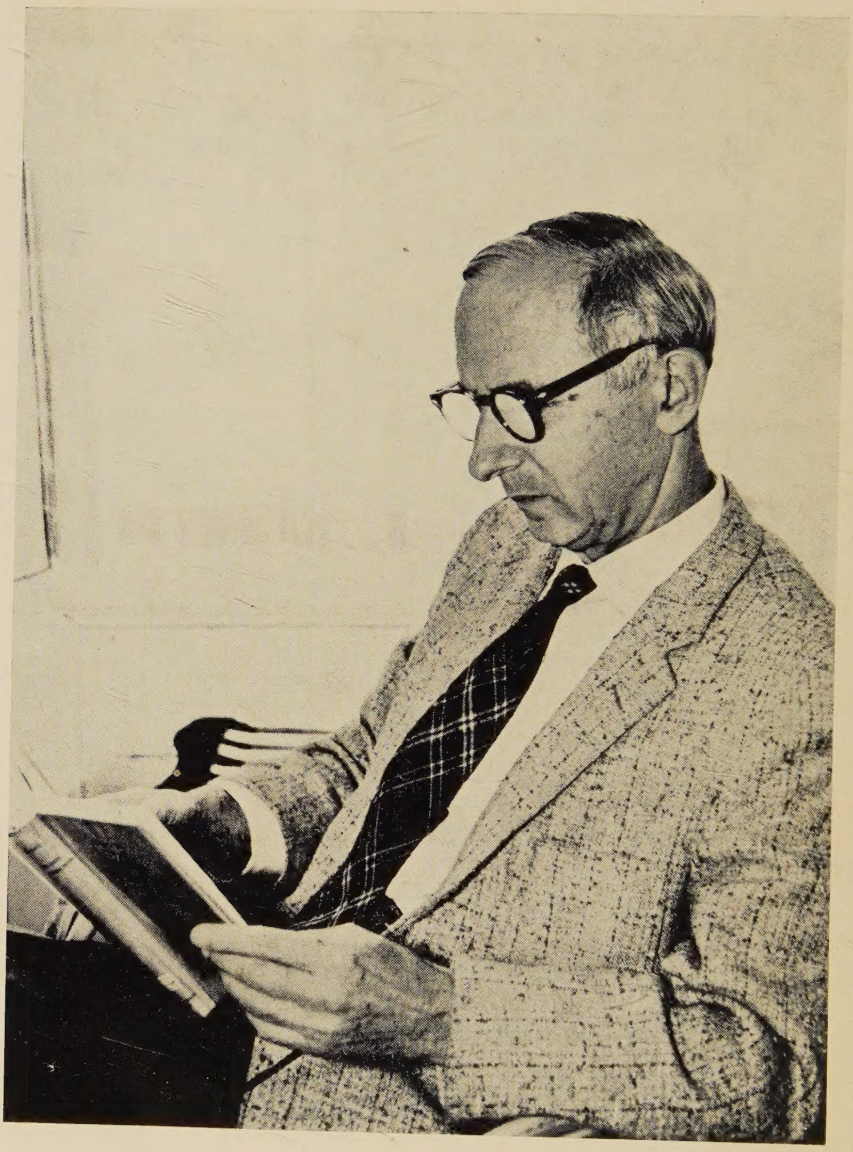

PROF. DR. T. H. VAN DEN HONERT

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Plant and Soil XII, no 1 January 1960

IN MEMORIAM
PROF. DR. T. H. VAN DEN HONERT

Unexpectedly for most of his friends, on the 10th May 1959 Prof. Dr. T. H. van den Honert, Consulting Editor of "Plant and Soil", passed away.

TACO HAJO VAN DEN HONERT was born on the 3rd June 1899 at Baarn, and studied biology at the State University of Utrecht. As a senior student he was especially interested in comparative physiology and in physico-chemistry, but finally he took his doctorate on a botanical subject.

In the laboratory of the late Prof. Dr. F. A. F. C. Went he developed a delicate method for measuring the photosynthesis of the green alga *Hormidium flaccidum* in a one-cell thick layer under strictly conditioned temperature, light intensity, and carbon dioxide tension. In this thesis he analysed the nature of the limiting factors in photosynthesis, and his considerations on the physico-chemical background of the limiting factors have retained their actuality to this day.

In 1928, soon after taking his D.Sc., van den Honert went to Java where he had been appointed plant physiologist at the Sugar Experiment Station, Pasuruan. Here his attention was focussed on the relation between plant and soil. By growing sugar cane on synthetic media he soon proved that a local failure of the crop ascribed to potassium deficiency was really due to the toxic effect of Fe^{++}-ions, the K-ions acting only as antagonists but not eliminating the cause of the trouble.

The Experiment Station disposed of the results of a very large number of field tests that had been instituted to study fertilizing with phosphate. Soil samples from the experiment fields had been

analysed on their phosphate content (citric acid extracts), and the sites of the experiments were indicated on soil maps of the plantations. However, for a number of soil types it proved to be impossible to correlate the analytical data with the results of the field tests. Van den Honert developed a method of cultivating sugar cane in a flowing nutrient solution, and by the aid of this method he determined accurately the uptake of water and of phosphate from the external solution. It appeared from a large number of experiments that the uptake of water and the uptake of phosphate are largely independent of each other. At very low phosphate concentrations phosphate can be absorbed at a rate 500 times higher than that of the water in which the phosphate had been dissolved.

Perhaps of still greater importance was van den Honert's discovery that only the univalent H_2PO_4-ion is taken up by sugar cane, whereas the bivalent HPO_4-ion and the trivalent PO_4-ion cannot be utilized. This finding could account completely for the lack of correlation found in a number of soil types between phosphate content and phosphate deficiency.

The quick growth of several large tropical fruits, which during the dry monsoon are able to store large amounts of water, offered a problem that was often discussed by the botanists of the Experiment Station, especially after Münch (1930) had published his theory on mass transport. Impressed by this theory van den Honert developed his idea on transport along boundary interfaces between hydrophilic and lipophilic phases of the protoplasm. This conception was illustrated by an elegant and convincing model, in which K-oleate migrates at a very high speed along a water – ether interface. One can argue, however, that none of the physiologically important compounds that are transported through the phloem, has – like K-oleate – a polar character, and that this is an indispensable condition of such a transport. For that reason van den Honert himself soon abandoned this idea. One aspect of it, however, seems still of importance. In his model van den Honert observed that the migrating K-oleate, by changing the surface tension of the boundary layer, dragged part of the two surrounding liquids in its track, thus causing a circulation in the water as well as in the ether phase. In the living protoplasm such a transport might be the moving force for the protoplasmic streaming. It is

possible, therefore, that this streaming is a consequence of the transport, whereas Hugo de Vries believed that it was the cause of the transport, which, as he pointed out, proceeds at a rate many times higher than can be accounted for by mere diffusion.

At the end of 1936 van den Honert left Pasuruan to take charge of the Treub Laboratory of the Government Botanical Gardens at Buitenzorg (Bogor), a laboratory, designed in the first place for guests from abroad, and as a centre for plant-physiological research. Here van den Honert studied transport problems in aerial roots of *Cissus*. After a few years, however, van den Honert became acting Director of the Botanical Gardens, and had to give up his own research, since all scientific institutes were involved in the governmental efforts to raise the defensive power of the Archipelago. Later on he was mobilized as captain of artillery and, after the Japanese invasion, he became a prisoner of war and was sent to one of the forced-labour camps in Siam concerned with the railroad construction through Burma. Van den Honert survived, but his health was in a very poor condition when Japan capitulated.

At the end of 1945 van den Honert was appointed professor of botany at Leyden University, which post he took up in the middle of 1946. Soon investigations were started in the field that had fascinated him already in Java: the uptake of ions by entire plants and by tissues, and the physico-chemistry of the involved mechanism. The latter, which is not subject to limiting factors, was compared with a conveyor belt, loading itself with ions at the boundary between protoplasm and external medium, and discharging its load somewhere inside the protoplast.

Since the readers of "Plant and Soil" will be familiar with the later publications of van den Honert and his students, special attention has been paid here to his early work, that sometimes has been published in the Dutch language and in less accessible periodicals.

Van den Honert was a man of culture and erudition, gifted with certain artistic talents, with good common sense and with a particular kind of humour. These properties has made him very popular among his many British friends, whom he regularly met at the meetings of the Society for Experimental Biology.

In 1955 and 1956 he stayed for a long time in the United States, and worked there in several laboratories. At this time the symptoms became apparent of a disease which gradually undermined his constitution. The early death of van den Honert means a heavy loss to his family and to his many friends.

Utrecht V. J. KONINGSBERGER

Plant and Soil XII, no. 1 Jannary 1960

DETERMINATION OF P STATUS OF *HEVEA BRASILIENSIS* BY BARK ANALYSIS

by H. W. VAN DER MAREL

Soil Survey Institute, Wageningen, The Netherlands

Symptoms of phosphorus deficiency are widespread in Hevea plantations in tropical latosols. They may easily be recognized by the stagnated growth, the small-sized, bronze- or purple-coloured leaves, the sparse foliage of the trees, and the bad flow of the concentrated latex.

The heavy rate of weathering in countries with high rainfall, which is a predominant factor in latex production, gives rise to leached P-deficient soils, which strongly fix the applied P manures. Especially when a pronounced dry season is absent, large amounts of moist, non-hydrated, active sesquihydroxides result from the decomposition of soil minerals. The velocity of P-fixation reaction is influenced by temperature as well as by concentration and pH. This points to a chemical process, although a Freundlich adsorption curve is obtained when P-fixing soils are shaken with increasing amounts of a phosphate solution van der Marel [28], Low and Black [27].

It is a common experience that when the above-mentioned latosols are treated with easily water-soluble phosphate (superphosphate), the crop will not respond to the applied manure, and P deficiency in the plantation will continue. Only after several years of heavy P applications followed by tapping on new bark will there be a noticeable response (see also Paul [36] and Owen [34]).

In alkaline soils (pH $>$ 8) the sesquiphosphates are unstable and they are decomposed to insoluble sesquihydroxides and soluble alkaliphosphates. Manuring with cheap, raw sesquiphosphates, so-called "hole phosphate" is successfully applied on marl soils at Java (see White and Hardon [53]).

The concentration of Al and especially of Fe in the soil solution decreases rapidly with the pH, along with the activity of these

ions to fix P ions. In soils with a pH > 6.5 fixation is due largely to divalent bases (Ca, Mg) and the availability (solubility products of the P compounds) is governed by pH, temperature and concentration of the divalent bases, and the CO_2 in the soil solution (see Buehrer [14] and Aslyng [1]. In soils of low calcium status there is a significant, direct correlation between the magnitude of P fixation and the amount of total active iron + aluminium = $= \frac{2.6 - SiO_2/Fe_2O_3 + Al_2O_3}{2.6}$ *.

It was found van der Marel [31], that Hevea plantations on tropical soils containing only some 0.001 per cent acid (7% HCl)-soluble P and which, moreover, fix up to 80 per cent of the P added (100 mg P_2O_5 as KH_2PO_4 on 20 g of soil), but which have more than 0.025 per cent organic P, do not show deficiencies.

Organic P in the soil is a component of humus, and results from plant debris and microbiological action. Its occurrence in soils was found as early as 1896 by Schmoeger [43]. Afterwards much experimental work was performed on this subject including the analytical way to determine this soil component (see Stoklasa [46], Potter and Benton [40], Schollenberger [44], *etc.*). Recently the literature on organic P has been summarized by Ulrich and Benzler [50].

According to Stoklasa [46] and Chaminade [15] organic P in soils exists mainly in the form of compounds of humus and phosphoric acid linked together by calcium (humophosphates). Bower [10 11] found that the NaOH extract of soils contained Ca, Mg, Fe and Al salts of phytinic acid, inosithexaphosphoric acid, and their derivatives. Its amount may be $\frac{1}{3}$ to $\frac{3}{4}$ parts of total organic P in soils. Other organic P compounds such as lecithin, nucleic acid, hexosephosphate and phosphoproteide are only believed to occur in negligible amounts as they are very liable to enzymatic action. Through weathering agents and microbiological activity organic P in the soil is converted to easily available P for the plant (see Neubauer [33], Bertramson and Stephenson [4], Pierre [39] and Picci [38]). In hot humid conditions this process is accelerated (see Eid *et al.* [22]) – and therefore the significance of organic P, which is gradually released as a P source to the plants in tropical soils, has increased.

---

* $\frac{SiO_2}{Fe_2O_3 + Al_2O_3}$ = the quotient for the amounts of SiO_2, Fe_2O_3 and Al_2O_3 in gram molecules which are extracted from the soil when it is heated for 1 hour at 55°C with HCl sp.w. 1.035 (van der Marel [28 30]).

According to van der Marel [29 31] and Owen [34 35], organic P in tropical latosols may amount to about 60 per cent of P extractable with strong acids at boiling temperature or of total P, the remainder being acid-soluble P, in several grades of acid concentration, exchangeable P and alkali-soluble sesquiphosphates.

Exchangeable P results from substitution of sesquihydroxide OH^- or lattice OH^-, or broken bond OH^- of clay minerals by PO_4^{---} (see Toth [48], Stout [47], Black [5], Kelly and Midglay [25], Turner and Rice [49], Weis *et al.* [52]) – and these kinds of P are also available to Hevea according to the experiments of Owen [34 35].

The anion-exchangeable OH^- in soils can be exchanged against F ions which have the same size (radius $OH^- = 1.45$ Å and radius $F = 1.33$ Å) (see the 0.1 *N* NH_4F extraction method of Dickman and Bray [21], Bray and Dickman [12] and Bray and Kurtz [13]). A greater response by Hevea to P manuring was found by Owen [34 35] when it was determined by the NH_4F extraction method instead of the weak-acid (0.002 *N* H_2SO_4) Truog method. However, the amount of anion-exchangeable P is low and in course of time this kind of P becomes more strongly bound.

Disordered, amorphous sesquiphosphates in soils are converted to well-ordered, micro-crystallized ones of various composition (see Scheffer and Schulz [42]). This reaction which takes time because of a low change in the free energy of formation (ΔF_T^0) is accompanied by an increase of the free energy of formation (F_T^0) of the component formed and therefore also by an increased resistance of the new component to the destructive action of weathering agents.

E.g. aluminium is more rapidly oxidized by oxygen than iron ($\Delta F^0_{298} Al_2O_3 = -376.8$ kcal mol^{-1}, $\Delta F^0_{298} Fe_2O_3 = -177.1$ kcal mol^{-1}) and the oxide formed is in the first case also more resistant than in the latter.

It should be emphasized that no universal chemical soil extract can determine exactly what is available to the plant in a soil. The factors determining this availability depend on so many factors (age of the plant, amounts and kinds of the available plant nutrients, weathering conditions during crop growth, mineral reserves of the soil type concerned, depth to which the roots penetrate, *etc.*). Especially in tropical soils the rate of weathering of minerals is a far more important factor in the supply of plant nutrients than in soils of moderate climates van der Marel [31].

Leaf composition of Hevea varies greatly with age, leaf type, leaf position, time of the day, *etc.*, just like that of apples, oilpalm, *etc.* (see for details about the composition of Hevea leaves Chapman [16], Bolle-Jones [6], Bolle-Jones and Ratnasingam [8] and Beaufils [3]. Just because of the unsatisfactory means of selection and collection of samples from mature trees, Bolle-Jones [9] also analysed bark samples of Hevea. It was found that the effect of P fertilizers in P-poor soils was more strongly demonstrated by the bark than by the leaves. Such in contrast to other plant nutrients (K, Mg) where bark analyses did not give better results. For N they were even worser. Biological procedures like the *Aspergillus niger* (Niklas), the seedling (Neubauer), or the weak-acid extraction procedures commonly used in moderate climates (Morgan, Truog, Dyer, Egnèr), have neither been found to be suitable for estimates of the P status of tropical soils (see van der Marel [31], Paul [36] and Owen [34 35]). The following are some analyses of rhyolitic soils from Hevea plantations of the East Coast of Sumatra and of the bark of these trees.

EXPERIMENTS

Soil analyses

The analyses of Table 1 show that the amount of humus is very low, especially in the subsoil – which is a common phenomenon in tropical latosols. Also N is low, specially in the subsoil. However, the latex of mature trees removes only 10 to 15 N kg per ha yearly and the trees (stem, twigs, bark, *etc.*) 25 to 30 kg N per ha. The N accumulated in the leaves is returned to the soil during 'winter' time each year.

Therefore only when the amount of N in these soils falls below 0.080 per cent (or below 0.060 per cent in the case of deep profiles) N deficiencies may occur (van der Marel [32]).

Sufficient potassium and magnesium is present in the investigated rhyolitic soils which contain biotite, a mineral weathering easily (van der Marel [31]). The same holds for calcium, although only small amounts of this plant nutrient occur because of the acid origin (rhyolitic mother rock) of the soils. Only about 10 kg K_2O, 0.6 to 0.8 kg MgO, and 0.5 to 0.7 kg CaO are removed by the latex of mature Hevea trees and the yearly uptake to form

TABLE 1

Soil analyses														
Soil sample No.	Depth in cm	$< 35\mu$ %	Humus %	N %	Dissolved by 25% HCl 1 hour 100°C				pH	1% citric acid %	P_2O_5 7% HCl * %	organic ** %	P-fixation %†	P organic
					K_2O %	CaO %	MgO %	P_2O_5 %						P 25% HCl
Block 3/1925														
53	5/20	32.1	3.09	0.149	0.065	0.032	0.095	0.018	5.4	0.002	0.007	0.014	30	0.78
54	35/55	48.5	0.68	0.035	0.132	0.009	0.195	0.002	4.7	0.002	0.001	0.002	51	1.00
55	5/20	46.2	3.97	0.178	0.027	0.021	0.048	0.020	5.2	0.002	0.007	0.014	30	0.70
56	35/55	55.5	0.82	0.045	0.019	0.018	0.045	0.005	4.7	0.002	0.002	0.001	44	0.20
Block 16/1937														
63	5/20	38.3	1.97	0.109	0.106	0.114	0.187	0.013	6.2	0.002	0.006	0.013	32	1.00
64	35/55	28.9	0.68	0.040	0.070	0.070	0.125	0.002	6.4	0.001	0.001	0.003	44	1.50
Block 6/1936														
59	5/20	49.3	4.07	0.160	0.072	0.064	0.150	0.021	5.3	0.002	0.007	0.019	36	0.91
60	35/55	63.6	0.68	0.034	0.080	0.029	0.140	0.004	4.9	0.001	0.003	0.002	57	0.50
Block 4/1937														
57	5/20	49.5	1.95	0.105	0.075	0.044	0.130	0.019	5.0	0.002	0.009	0.021	25	1.11
58	35/55	56.4	0.67	0.043	0.064	0.012	0.118	0.010	4.8	0.001	0.004	0.006	41	0.60
Block 11/1937 (alluvial)														
61	5/20	56.8	2.34	0.121	0.140	0.055	0.182	0.050	5.2	0.012	0.037	0.026	25	0.52
62	35/55	78.2	1.13	0.066	0.085	0.027	0.110	0.028	4.9	0.002	0.020	0.010	38	0.36

* P_2O_5 = P_2O_5 dissolved by 7% HCl for 1 hour at 55°C.

** P_2O_5 organic = P_2O_5 destructed by H_2O_2 – Marel [28].

† P-fixation = P_2O_5 fixed by 20 g of soil from 250 cc KH_2PO_4 solution in which 100 mg P_2O_5, for 24 hours at 29 30°C.

Note: Block no. 11 is a white-gray alluvial soil; the other soils are brown or brown-red coloured latosols.

new twigs, stems and bark is of the order of only 20 to 35 kg K_2O, 10 to 15 kg MgO, and 25 to 40 kg CaO.

The fluctuations in adsorbed plant nutrients depend on the type of soil where Hevea is grown. Thus mature Hevea leaves grown on K-rich but Ca-deficient rhyolitic soils have, when compared with those grown on more basic soil types, relatively higher amounts of potassium – about 1.5 to 2 per cent K_2O against 0.8 to 1.2 per cent K_2O – and low amounts of calcium – about 0.8 to 1 per cent CaO against 1.5 to 2.2 per cent CaO. Magnesium is less variable, *i.e.* about 0.5 to 0.6 per cent against 0.6 to 0.8 per cent MgO, because the rhyolitic soils contain more plant-available magnesium than calcium and sometimes more magnesium than potassium (see for K/Ca and K/Mg antagonism of Hevea: Chapman [16], De Haan [19], Bolle-Jones [6 7], Rhines *et al.* [41], and Beaufils [3].

Hevea needs only about 10 to 15 kg P_2O_5 per ha yearly for the production of new twigs, stem, roots and bark and mature trees remove about 6 to 10 kg P_2O_5 per ha from the soil in the latex. The yearly leaf fall (wintering, at the beginning of the dry season) returns the P accumulated in the leaves to the soil.

It was found (van der Marel [31]) that when P_2O_5 inorganic dissolved by 7% HCl at 55°C is practically absent (0.002 to 0.010 per cent) and organic P is < 0.010 per cent, P manuring may be successfully applied. However, between the narrow range of 0.010 to 0.025 per cent P_2O_5 no clear distinction exists between non-responsive and responsive soils. The reason is that besides organic P there may also be anion-exchangeable P which is also available to Hevea (see the experiments of Owen [34 35]).

A further difficulty arises from the observation that soils with deep, well-aerated profiles, where Hevea roots penetrate to several metres depth, may contain smaller amounts of organic P per gram of soil than soils with shallow profiles (alluvial or peaty soils) with a high water table. Consequently the interpretation of soil analyses in the 0.010 to 0.025 per cent organic P_2O_5 range may be very complicated, as more factors must be considered.

The analyses show that P_2O_5 inorganic in the soils investigated is very low as could be expected from their acid parent rock (rhyolite) origin. Only the alluvial soil is an exception. This soil contains sufficient organic P and, moreover, there is moderate P fixation in the top soil. Here the P-rich topsoil from the surrounding more

elevated regions has accumulated. The other soils have only a small amount of P organic but, from the amounts found, it could not be concluded whether they should be classified as P-responding or non-responding soils. Moreover the soils fix appreciably amounts of P and they are acid.

From farming experience on the above brown and red-brown latosols and the gray-white alluvial soils, it is known that only the red-brown latosols need P manure – about 200 to 300 kg fine Algeria phosphate per ha yearly.

Only where the alluvial soils are very coarse and therefore contain only small amounts of humus and therefore also organic P, phosphate manure is needed. The same contrast in behaviour of these both soil types is also found at Malacca (see Owen [34 35]), where the soils are also derived from P-deficient parent rock (granite, shales, *etc.*).

Bark analyses

The barks were sampled with a common laboratory cork bore of one inch diameter. The bore was inserted into the stem at a height of about 125 cm down to the wood. Each bark sample consisted of 15 to 18 bark sections bored from 5 to 6 neighbouring Hevea stems situated around a certain sample place in the plantations from which also the soil samples were taken.

From the analyses – see Table 2 – it follows that in contrast with the wide differences in K_2O, CaO, and MgO of the soils, the composition of the barks is less variable; even in spite of the various sorts of Hevea trees. This is especially true when the amounts are fixed at 100 total me $K_2O + CaO + MgO$. Real percentages have more fluctuations, as the total amount of $K_2O + CaO + MgO$ is influenced by age, the younger plant material having the largest total amount. This effect is especially clear for leaves with a pronounced cycle (apples, potatoes) and it is mostly absent for grains and tubers, which tend to have a constant composition.

It may furthermore be concluded from Table 2, that the analyses of barks from the same blocks, but sampled at another date also give practically the same results for K_2O, CaO and MgO when expressed in me $K_2O + CaO + MgO$. No large variations were found in the nitrogen content of the barks from several soils sampled at different dates.

TABLE 2

Bark analyses										
Block	Kind of tree	K_2O %	CaO %	MgO %	$K_2O+CaO+MgO$, me	On total me $CaO+MgO+K_2O$			N %	P_2O_5 %
						K_2O %	CaO %	MgO %		
Date of sampling: 28/6–1956										
3/1925	Graft *	0.57	3.49	0.34	153.6	7.9	81.1	11.0	0.28	0.058
	Seedling *	0.59	3.67	0.43	164.9	7.6	79.5	12.9	0.34	0.068
	Graft *	0.75	3.47	0.41	160.1	9.9	77.4	12.7	0.42	0.092
	Seedling *	0.72	3.62	0.37	163.0	9.4	79.3	11.3	0.36	0.076
16/1937	Av 255	0.71	3.78	0.35	167.5	9.0	80.6	10.4	0.38	0.078
6/1936	Av 49	0.95	4.40	0.33	193.7	10.4	81.1	8.5	0.41	0.119
4/1937	R 1124	0.88	2.98	0.39	144.4	12.9	73.7	13.4	0.44	0.143
11/1937 **	LCB 510	0.93	3.38	0.50	165.2	11.9	73.0	15.0	0.36	0.316
Date of sampling: 28/12–1955										
3/1925	Graft *	0.45	3.40	0.48	154.7	6.1	78.5	15.4	0.28	0.048
	Seedling *	0.50	3.70	0.48	166.5	6.4	79.3	14.3	0.27	0.061
	Graft *	0.48	2.50	0.30	114.4	8.9	78.1	13.0	0.27	0.085
	Seedling *	0.53	3.70	0.34	160.2	7.0	82.5	10.5	0.27	0.072
6/1936	Av 49	0.72	2.85	0.28	131.0	11.7	77.7	10.6	†	†
11/1937 **	LCB 510	0.70	2.48	0.43	124.8	11.9	71.0	17.1	†	†

* = Further details not available ** = Alluvial † = Sample insufficient for analyse

On the other hand the barks of the various soils contain quite different amounts of P. The P-rich alluvial soil (Block 11) has about 3 to 5 times as much P as that of the very poor Blocks 3 and 16. The order in which increasing amounts of P in the barks are found, *i.e.* 3/1925, 16/1937, 6/1936, 4/1937, 11/1937, is the same as that in which the soils concerned contain increasing amounts of organic P. The divergence in the organic P amounts is, however, much smaller than that in the amounts of P in the barks. When the barks were sampled at another date, practically the same amounts for P were found (see Table 2).

DISCUSSION

Beaufils [3] analysed leaves and latex of various *Hevea* clones at different dates in the year. The samples came moreover from trees grown on different soil types which were treated with several kinds of manure.

It was found that mineral composition of latex is less variable than that of the leaves. Thereby interclonal variation in P content of latex was small (see also Philpott and Westgarth [37]). The narrow relationship which exists between the amount of available P in the soil and the P content of the bark may be ascribed to the indispensability of energy-rich, phosphorylated compounds for the transfer of energy during the synthesis of carbohydrates, amino acids, peptides, protein, lipo-proteins, *etc.* (see Lipman [26], Kalcker [24], Baudisch [2], Webster [51]).

In this case these phosphorylated compounds, for example A.T.P. (adenosine-triphosphoric acid), should stimulate the synthesis of the caoutchouc (polypren) particles which occur in the latex to an amount of 28 to 40 per cent. Copper ions are well known for their activating action on enzymes (see Haehn and Schweigart [23] and Schweigart [45]). Its injection into the rubber tree stimulated latex production (see Compagnon and Tixier [17], Compagnon and Beaufils [18], Beaufils [3]). According to Beaufils [3] and De Jonge [20] an increase in latex yield caused by $CuSO_4$ or Stimulex (an organic stimulant = 2,4-dichlorophenoxy-acetic acid) runs parallel to an increase in the amount of P and Cu respectively in the latex and a decrease of these elements in the leaves.

Therefore if copper ions are added, the potential difference between two existing redox systems in the plant cell may be raised and consequently the activity of the enzymes which are concerned with the synthesis of caoutchouc particles from carbohydrates by degradation, may be significantly increased.

SUMMARY

The several methods to determine the P status of *Hevea Brasiliensis* on tropical soils are reviewed. The matter is very complicated, as several P compounds are found in soils, *i.e.* organic P, acid soluble P, exchangeable P, alkali-soluble P and inert P. It was found that P analyses of Hevea barks can better represent the amount of available P in the soil than soil analyses. Bark analyses are, moreover, less markedly influenced by date of sampling than leaf analyses. This characteristic feature of P in barks of Hevea may be ascribed to the outstanding property of energy-rich phosphorylated compounds as agents in increasing the synthesis of caoutchouc particles from the daily assimilates and the carbohydrate reserves.

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REFERENCES

1 Aslyng, H. C., The lime and phosphate potentials of soils; the solubility and availability of phosphates. Arsskrift Den. Kgl. Vet. og Landbohøgskole **7**, 1–50 (1954).
2 Baudisch, O., Biological function of minor elements. Soil Sci. **60**, 173–184 (1945).
3 Beaufils, E. R., Recherches sur la variation des constituants minéraux dans les feuilles et le latex de l'Hevea brasiliensis. Rev. Gén. Caoutchouc **32**, 323–331 (1955).
4 Bertramson, B. R. and Stephenson, R. E., Comparative efficiency of organic phosphorus and of superphosphate in the nutrition of plants. Soil Sci. **53**, 215–227 (1942).
5 Black, C. A., The penetration of phosphate into the kaolinite crystal. Soil Sci. Soc. Am. Proc. **6** (1941), 157–161 (1942).
6 Bolle-Jones, E. W., Effect of nutritional deficiencies on growth, chlorophyll, rubber and mineral contents of Tjirandji seedlings. J. Rubber Research Inst. Malaya **14**, 209–230 (1954).
7 Bolle-Jones, E. W., The interrelationships of magnesium, potassium and phosphorus. J. Rubber Research Inst. Malaya **14**, 231–256 (1954).
8 Bolle-Jones, E. W. and Ratnasingam, K., Interclonal and seasonal variations in composition of leaves. J. Rubber Research Inst. Malaya **14**, 257–275 (1954).
9 Bolle-Jones, E. W., Foliar diagnosis and bark analysis of Hevea. J. Rubber Research Inst. Malaya **15**, 109–127 (1957).
10 Bower, C. A., Separation and identification of phytin and its derivatives from soils. Soil Sci. **59**, 277–285 (1945).
11 Bower, C. A., Studies on the form and availability of soil organic phosphorus. Iowa Agr. Expt. Sta. Research Bull. **362** (1949).
12 Bray, R. H. and Dickman, S. R., Adsorbed phosphates in soils and their relation to crop responses. Soil Sci. Soc. Am. Proc. **6**, 312–320 (1942).
13 Bray, R. H. and Kurtz, L. T., Determination of total organic and available forms of phosphorus in soils. Soil Sci. **59**, 39–45 (1945).
14 Buehrer, T. F., The physico-chemical relationships of soil phosphates. Ariz. Agr. Expt. Sta. Tech. Bull. **42**, 154–212 (1932).
15 Chaminade, R., Les formes du phosphore dans le sol: Nature et rôle des complexes phospho-humiques. Ann. Agron. **14**, 1–53 (1944).
16 Chapman, G. W., Leaf analyses and plant nutrition. Soil Sci. **52**, 63–81 (1941).
17 Compagnon, P. and Tixier, P., Sur une possibilité d'améliorer la production d'Hevea brasiliensis par l'apport d'oligo-éléments. Rev. Gén. Caoutchouc **27**, 525–526, 591–594, 663–665 (1950).
18 Compagnon, P. and Beaufils, E. R., Sur l'activité du cuivre en tout qu'oligoélément dans l'assimilation minérale de l'Hevea. Compt. Rend. **240**, 1493–1495 (1955).
19 De Haan, I., Deficiency symptoms in Hevea Brasiliensis. Arch. Rubbercult. Ned. Indië **27**, 107–137 (1950).
20 De Jonge, P., Further observations on the effect of yield stimulants. J. Rubber Research Inst. Malaya **14**, 383–406 (1955).

21 Dickman, I. R. and Bray, R. H., Replacement of adsorbed phosphate from kaolinite by fluoride. Soil Sci. **52**, 263–273 (1941).
22 Eid, M. T., Black, C. A., and Kempthorne, O., Importance of soil organic phosphorus to plant growth at low and high soil temperatures. Soil Sci. **71**, 361–370 (1951).
23 Haehn, H. and Schweigart, H. A., Potato amylase separation into neutral salts and anorganic components. Biochem. Z. **143**, 516–526 (1923).
24 Kalcker, H. M., The nature of energetic coupling in biological synthesis. Chem. Rev. **28**, 71–78 (1941).
25 Kelly, J. B. and Midglay, A. R., Phosphate fixation – an exchange of phosphate and hydroxyl ions. Soil Sci. **55**, 167–176 (1943).
26 Lipman, F., Metabolic generation and utilization of phosphate bond energy. Advances in Enzymol. **1**, 99–162 (1941).
27 Low, P. F. and Black, C. A., Phosphate induced decomposition of kaolinite. Soil Sci. Soc. Am. Proc. **12**, 180–184 (1948).
28 Marel, H. W. van der, Cation and anion adsorption of tropical and Dutch soils. Thesis Agr. Univ. Wageningen (1935).
29 Marel, H. W. van der, Organic phosphorus in soils. Landbouwk. Tijdschr. **47**, 85–88 (1935).
30 Marel, H. W. van der, Phosphate fixation in soils. Landbouwk. Tijdschr. **48**, 496–503 (1936).
31 Marel, H. W. van der, Tropical soils in relation to plant nutrition. Soil Sci. **64**, 445–451 (1947).
32 Marel, H. W. van der, Humus as a regulator of water and nitrogen status of tropical soils. Landbouwk. Tijdschr. **60**, 115–120 (1948).
33 Neubauer, E., Versuche nach der Keimpflanzenmethode über den Unterschied der Aufnehmbarkeit anorganischer- und organischer Phosphatformen. Landwirtsch. Vers. Sta. **114**, 225–294 (1933).
34 Owen, G., Determination of available nutrients in Malayan soils. J. Rubber Research Inst. Malaya **14**, 109–120 (1953).
35 Owen, G., Studies on the phosphate problem in Malayan soils. J. Rubber Research Inst. Malaya **14**, 121–132 (1953).
36 Paul, H., Some observations on the mechanism of phosphorus fixation in some soils of British Guiana. J. Agr. Sci. **39**, 249–253 (1949).
37 Philpott, M. W. and Westgarth, D. R., Stability and mineral composition of Hevea latex. J. Rubber Research Inst. Malaya **14**, 133–148 (1953).
38 Picci, G., Die Mineralisation von organischen Phosphor durch Microben referated in Chem. Zentr. 1954, vol. 125, no 5157 (1954).
39 Pierre, W. H., The phosphorus cycle and soil fertility. J. Am. Soc. Agron. **40**, 1–14 (1948).
40 Potter, R. S. and Benton, T. H., The organic phosphorus of soil. Soil Sci. **2**, 291–298 (1916).
41 Rhines, C. E., McGavack, J., and Linke, C. J., Mineral nutrition of Hevea brasiliensis. Rubber Age N.Y. **70**, 467–474 (1951).
42 Scheffer, F. and Schulz, H. G., Darstellungsbedingungen und Eigenschaften einiger Eisenphosphate: Ein Beitrag zur Phosphatfestlegung im Boden. Z. Pflanzenern. Düng. u. Bodenk. **70**, 141–164 (1955).
43 Schmoeger, M., Untersuchen über einige Bestandteile des Moores. Landwirtsch. Jahrb. **25**, 1025–1050 (1896).
44 Schollenberger, C. J., Organic phosphorus of soil: Experimental work on methods for extraction and determination. Soil Sci. **6**, 365–395 (1918).

45 Schweigart, H. A., Integrierende Spurenelemente in biologischen Systemen. Z Pflanzenern. Düng. u. Bodenk. **54**, 36–58 (1951).
46 Stoklasa, J., Biochemischer Kreislauf des Phosphations im Boden. Centr. Bakteriol. Parasitenk. Abt. II **29**, 358–519 (1911).
47 Stout, P. R., Alterations in the crystal structure of clay minerals as a result of phosphate fixation. Soil Sci. Soc. Am. Proc. **4**, 177–183 (1940).
48 Toth, S. J., Anion adsorption by soil colloids in relation to changes in free iron oxides. Soil Sci. **44**, 299–314 (1937).
49 Turner, R. C. and Rice, H. M., Role of the fluoride ion in release of phosphate adsorbed by aluminium and iron hydroxides. Soil Sci. **72**, 141–148 (1952).
50 Ulrich, B. and Benzler, J. H., Der organisch gebundene Phosphor im Boden. Z. Pflanzenern. Düng. u. Bodenk. **70**, 220–249 (1955).
51 Webster, G. C., Nitrogen metabolism. Ann. Rev. Plant Physiol. **6**, 43–70 (1955).
52 Weiss, A., Mehler, A., Koch, G., and Hofmann, U. Über das Anionen Austauschvermögen der Tonmineralien. Z. Anorg. u. Allgem. Chem. **284**, 247–271 (1956).
53 White, J. Th. and Hardon, H. J., Potproeven met inheemsch natuurlijk aluminiumphosphaat als meststof. Korte Meded. Proefst. Landbouw, Buitenzorg, Ned. Indië **no 5** (1935).

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THE DISTRIBUTION AND CONDITION OF SOIL PHOSPHATE UNDER OLD PERMANENT PASTURE

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INTRODUCTION

Knowledge of the residual value of phosphatic fertilisers under different soil, climatic, and cropping conditions is very limited. This is particularly true in the case of phosphatic dressings which have been applied over a long period of time and have been subjected to fixation and other soil reactions for many years. The Tree Field Experiment situated at Cockle Park, Northumberland, was an ideal site for the study of this problem as it had received the manurial treatments shown in Table 1 from 1897 until 1954.

TABLE 1

The manurial treatments of the Tree Field experiment for a six-year cycle	
Plot	Manure applied (per acre)
1	300 lb. high-protein cake fed annually to sheep for first 3 years only.
2	3.175 cwt. mineral phosphate (100 lb. phosphoric acid) every 3rd year.
3	10 cwt. basic slag (200 lb. phosphoric acid) every 6th year.
4	5 cwt. basic slag (100 lb. phosphoric acid) every 3rd year.
5	Superphosphate supplying 100 lb. phosphoric acid every 3rd year.
6	Control plot – no treatment.
7	Slag as plot 4: 100 lb. muriate of potash (60% K_2O) every 3rd year.
8	Slag as plot 4: 1 ton common lime every 3rd year.
9	Slag as plot 4: 1 cwt. nitrate of soda every 3rd year.
10	609 lb. dissolved bones (100 lb. phosphoric acid) every 3rd year.

When the original trial was terminated in 1954 it was decided to estimate the residual value of the fertiliser treatments by measuring the growth and yield of two cereal crops. As a preliminary to this a comprehensive soil-sampling programme was carried

out. Pedological examination of the soil revealed it to be a heavy clay loam developed on boulder clay overlying millstone grit. The soil was gleyed at depths of 10 to 18 inches and further evidence of impeded drainage was provided by rusty mottling very near the surface on most plots. This type of soil is representative of large areas of northern England.

Roy and Thomas [19] had shown that large quantities of phosphate were present in the soil of Tree Field in 1948, but subsequent pot experiments involving the growth of radish (*Raphanus sativa* L.) did not substantiate the data obtained from chemical analyses of the soil. It was hoped that the new programme of sampling and analyses together with the growth and yield measurements of the cereal crops would give a valid estimation of the residual value of the phosphate dressings.

EXPERIMENTAL METHODS

Six original plots were selected for the purposes of the new trial these being plots 4, 5, 6, 7, 8, and 9. Before the sward was ploughed a soil map of the experimental site was prepared. Two profile pits were then excavated on each of the selected plots and soil samples taken at 3-inch intervals down the profile to a depth of 24 inches and then a further sample was taken at 36 inches. The samples were air-dried, ground to pass a 2-mm sieve and subjected to chemical analyses. pH was determined by a Cambridge pH meter. Total phosphorus was determined by extracting the soil with 60% perchloric acid A.R. then measuring the colour intensity produced in the extract by ammonium vanado-molybdate reagent in a Spekker photoelectric absorptiometer. Available phosphorus was estimated by shaking the soil with Morgan's solution, oxidising the extract with persulphate reagent and measuring the colour intensity produced in the extract by ammonium molybdate reagent in a Spekker photoelectric absorptiometer. Available calcium was determined from the same extract by adding ammoniacal citrate and dilute soap solution and comparing against known standards.

TABLE 2

The fertilisers applied in 1956 and 1957

Type of fertiliser	Rate of application cwt./acre/year	Nutrients applied per acre per year
Nitro-chalk (15.5% N)	2	34.8 lb. N
Triple-superphosphate (47% P_2O_5) . .	1	53.7 lb. P_2O_5
Muriate of potash (60% K_2O)	2	134.4 lb. K_2O
Hydrated lime (75% CaO)	28 *	21 cwt. CaO *

* Applied prior to 1956 oat crop only.

The sward was ploughed in October 1955 and Sun II oats were sown at 196 pounds per acre on April 16th, 1956. A full combination of the fertilisers shown in Table 2, together with controls receiving no fertiliser were replicated twice on each original plot. The fertilisers were applied to the seed-bed prior to sowing. This was repeated before Svenno wheat was sown on April 12th 1957 at 168 pounds per acre with the exception of the hydrated-lime dressing which was only applied prior to the oat crop in 1956.

The yield from each sub-plot was determined from an area 8 feet wide and 45 yards long which was harvested by a self-propelled combine harvester.

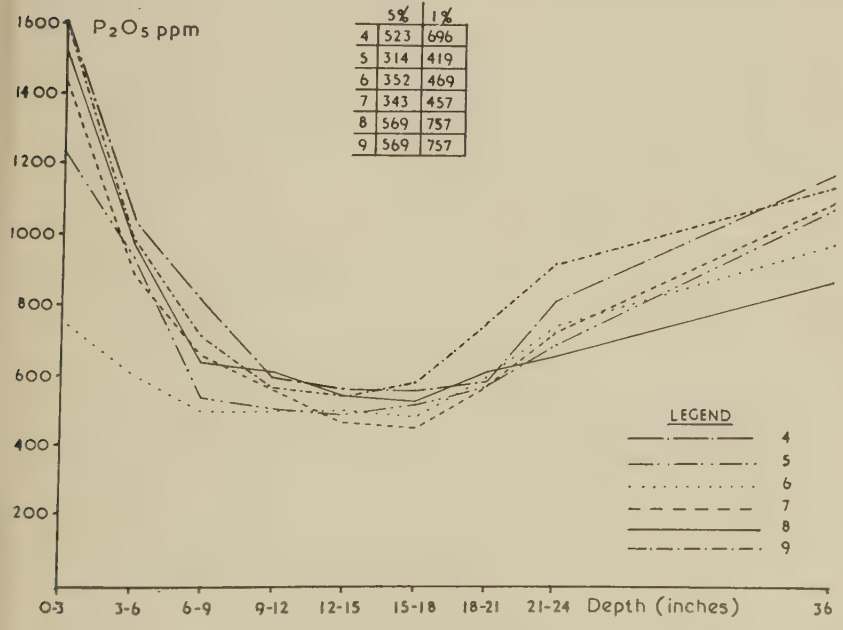
RESULTS

The following chemical analyses of soil were carried out prior to ploughing:

1. *Total phosphorus*

The total-phosphorus contents of the various strata are given in Table 3 and illustrated in Figure 1.

There was a variation in the mean total-phosphate contents of the original plots, plot 6 having the lowest mean total-phosphate



	5%	1%
4	523	696
5	314	419
6	352	469
7	343	457
8	569	757
9	569	757

Fig. 1. Total-phosphate contents of the plots 4 to 9 incl. at various depths.

TABLE 3

Total phosphate (ppm P_2O_5). Tree Field soil 1954							
Depth (inches)		Original plot					
		4	5	6	7	8	9
0–3		1635	1237	744	1441	1522	1613
3–6		1033	937	605	880	963	982
6–9		816	555	513	661	643	711
9–12		589	497	491	487	608	534
12–15		544	468	493	443	536	535
15–18		539	497	457	420	512	562
18–21		567	563	586	569	599	749
21–24		807	689	748	737	667	905
36		1156	1037	981	1051	855	1069
Mean		961	810	701	836	863	958
Difference between horizons in original plot	$P=5\%$	523	314	352	343	569	569
	$P=1\%$	696	419	469	457	757	757

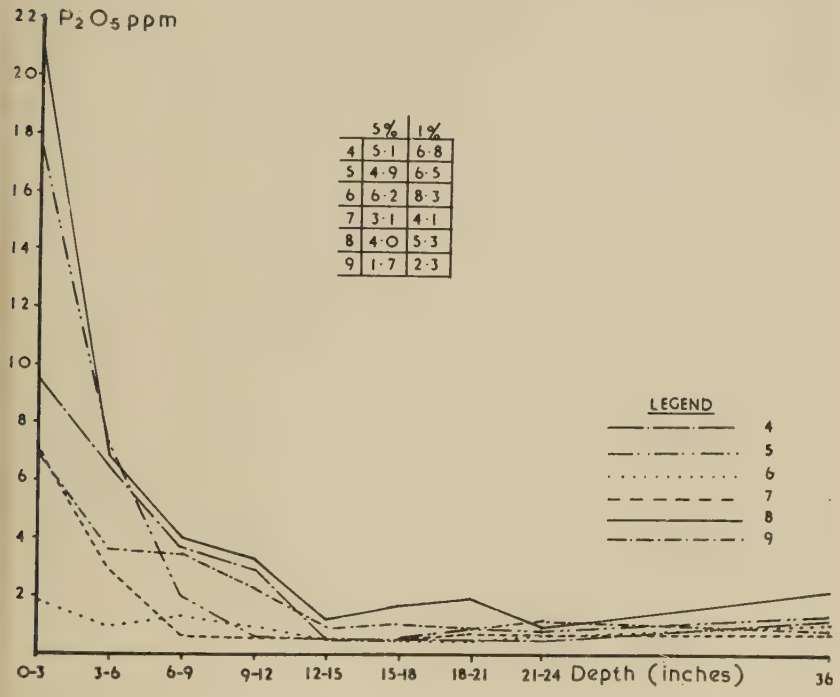
content. The distribution of total phosphate in the soil profiles showed that plots 4, 8, and 9 were similar in that the 0- to 3-inches stratum had a significantly higher total-phosphate content than any other depth. Plots 5 and 7 had high levels of total phosphate in the surface layers but also contained significant amounts at 3 to 6 inches and at 36 inches. There was no surface concentration of total phosphate on plot 6 where the phosphate content of soil from a depth of 36 inches exceeded that in the 0- to 24-inches strata.

TABLE 4

Available phosphate (ppm P_2O_5). Tree Field soil 1954							
Depth (inches)		Original plot					
		4	5	6	7	8	9
0–3		9.5	17.5	1.9	7.1	20.7	7.2
3–6		3.7	6.6	1.1	2.8	5.2	3.7
6–9		2.9	2.0	1.2	0.5	3.1	3.4
9–12		0.5	0.5	0.8	0.5	1.1	1.1
12–15		0.5	0.5	0.5	0.5	1.1	0.8
15–18		0.5	0.5	0.5	0.5	1.5	1.3
18–21		0.5	1.1	0.5	1.1	1.7	1.1
21–24		0.5	1.1	0.8	1.1	0.8	1.5
36		1.7	1.6	1.2	0.8	2.1	1.1
Mean		2.3	3.5	1.2	1.7	4.2	2.4
Difference between horizons in original plot	$P=5\%$	5.1	4.9	6.2	3.1	4.0	1.7
	$P=1\%$	6.8	6.5	8.3	4.1	5.3	2.3

2. *Available phosphate*

The available-phosphate results are given in Table 4 and Figure 2. Plots 5 and 8 had the highest mean available-phosphate contents while plots 4 and 7 were intermediate in this respect and plot 6 comparitively lacking in available phosphorus.



	5%	1%
4	5·1	6·8
5	4·9	6·5
6	6·2	8·3
7	3·1	4·1
8	4·0	5·3
9	1·7	2·3

Fig. 2. Available-phosphate contents of the plots 4 to 9 incl. atvarious depths.

When the plots were considered individually the results followed a definite pattern. On every plot, except 6, the 0 to 3-inches stratum contained levels of available phosphate which were highly significant as compared to those in other strata. In addition, plots 5 and 8 had significant amounts of available phosphorus in the 3- to 6-inches stratum. On all the plots strata between depths of 9 and 21 inches contained reduced levels of available phosphate but increased levels were present at 36 inches with the exception of plot 7.

3. *pH*

The distribution of pH in the soil profiles, given in Table 5,

TABLE 5

pH. Tree Field soil 1954							
Depth (inches)		Original plot					
		4	5	6	7	8	9
0–3		5.6	5.6	5.4	6.2	7.5	6.4
3–6		5.9	5.3	5.6	6.0	7.5	6.4
6–9		6.3	5.6	6.2	6.2	7.7	6.6
9–12		6.6	5.9	6.7	6.4	7.7	6.7
12–15		6.8	6.1	6.8	6.7	7.7	7.0
15–18		7.0	5.8	7.1	6.9	7.8	7.2
18–21		7.1	6.3	7.1	7.0	7.9	7.3
21–24		7.3	6.7	7.3	7.2	7.8	7.4
36		7.7	7.3	7.7	7.8	7.8	8.0
Mean		6.7	6.0	6.6	6.7	7.7	7.0
Difference between horizons in original plot	$P=5\%$	0.4	0.8	0.8	0.7	0.5	0.5
	$P=1\%$	0.5	1.1	1.1	0.9	0.6	0.6

contrasted with that for both total and available phosphate in that the highest values were found in the lowest strata. Differences, although apparently small, proved to be statistically significant except on plot 8. It was notable that the mean pH value of 6.0 on plot 5 receiving superphosphate was below that of the remaining plots including plot 6. Plot 8 had a relatively high pH value of 7.7 but in no instance could the pH be described as being low especially in view of the type of soil.

TABLE 6

Available calcium (ppm). Tree Field soil 1954							
Depth (inches)		Original plot					
		4	5	6	7	8	9
0–3		3600	2400	1800	3800	8500	3000
3–6		2800	1800	2000	2400	8000	3400
6–9		2800	2400	3200	2600	6000	2800
9–12		3200	2400	2800	2600	4000	3200
12–15		2600	2400	3200	2200	4000	4300
15–18		3200	3000	3400	2200	4000	5800
18–21		3400	3400	3400	2800	7000	5300
21–24		3600	3000	4000	4300	7000	5000
36		6100	6100	10000	7500	6100	10000
Mean		3500	3000	3800	3400	6100	4800
Difference between horizons in original plot	$P=5\%$	5710	6214	3761	5638	11974	4240
	$P=1\%$	7600	8271	5007	7505	15821	5643

4. *Available calcium*

As would be expected the distribution of available calcium resembled that of pH in that the highest concentrations were found in the lowest strata. However, as can be seen in Table 6, plot 8 was an exception to this as the available calcium content of the surface 9 inches exceeded that found at 36 inches. In contrast the available calcium contents in the 36-inches horizon on plots 6 and 9 were significantly greater than at any other depth. The available calcium contents of plots 4, 7, and 9 which had received dressings of basic slag were all above that of plot 5 receiving superphosphate.

DISCUSSION

The distribution of phosphorus in Tree Field soil

The pattern of the distribution of total phosphorus in the soil profiles was similar on all the plots. Although the untreated plot 6 had less total phosphate in the surface layers than other plots, all plots had relatively equal amounts of total phosphate in the 9- to 12-inches stratum. This indicates that the phosphatic fertilisers applied as topdressings had only penetrated to a depth of approximately 9 inches during a period of 58 years. This agrees with Roy [18] who found that between 85 and 98 per cent of the phosphate applied to Tree Field could be accounted for in the surface 11 inches. Rice Williams [17] and Doak [9] also report limited movement of phosphate applied as topdressings to permanent pasture. Results from investigations examining surface applications of phosphatic fertilisers to permanent pasture have been in agreement with the results from Tree Field where the liveweight gains of grazing animals rose by 400 per cent following the fertiliser dressings [5]. Nelson *et al.* [16] and Lawton *et al.*[13] have reported large responses from fertilisers applied in this manner and data obtained with radioactive phosphatic fertilisers by Stanford *et al.*[20] and Caldwell *et al.* [4] has shown that established pasture will readily utilise phosphate applied as topdressings. It would appear therefore that the restricted movement of phosphate applied as topdressings does not reduce the effects of the fertiliser.

The restricted movement of phosphate was even more pronounced in strata immediately below 12 inches. The total-phosphate content remained relatively constant on each plot between depths of 12 to

18 inches. Roy [18] considered that the reduced amount of phosphate at these depths was due largely to the removal of the nutrient by the sward, which in the absence of downward movement, was not replaced. A factor which may have been equally important, however, was the marked degree of gleying found at these depths. Soil between depths of 12 and 18 inches contained only small amounts of available phosphate, as shown in Table 4, suggesting that a factor common to all plots was causing phosphate to be present in an unavailable condition. Gleyed horizons are usually low in available phosphate [10] and it is possible for phosphate to become mobile under these conditions [15]. Phosphate may have moved out of the gleyed horizons and this may account for the larger amounts of total and available phosphate found in the soil at greater depths. Large prismatic structures were present in the gleyed horizons and the roots of the sward were restricted to the surfaces of these structures. These factors together with the gleyed conditions suggest that the sward would be unable to utilise phosphate in the horizons between depths of 12 to 18 inches very effectively.

The total-phosphate contents of soil in the 36-inches stratum were comparable to those at a depth of 3 to 6 inches on all plots and on plot 6 even exceeded the concentration found in the surface layers. Several workers [1 21] have found that the distribution of total phosphate in lower soil strata may be important and Crowther [6] has shown that it is possible for crops to utilise nutrients in lower horizons under favourable conditions. There was more phosphate in available form at 36 inches than in strata between depths of 12 and 24 inches on all plots and the roots of the sward were surprisingly profuse and well developed at this depth. The problem of available phosphate in lower strata is one of positional availability, but it is known that grasses can assimilate phosphate at depths of 36 inches [24]. It is possible that phosphate at this depth on Tree Field was utilised by the sward and may have contributed towards the increased productivity.

The availability of phosphorus in Tree Field soil

The presence of large quantities of total phosphate in the surface soil of Tree Field can have had little significance if the phosphate was not available to plants. The available-phosphate determinations at various depths, given in Table 4 and Figure 2, illustrated that

plot 5 receiving superphosphate and plot 8 receiving basic slag and lime contained more available phosphate in the surface layers than other plots. When the available phosphate was expressed as a percentage of the total phosphate as in Table 7, it was found that plots 5 and 8 had a much higher proportion of the total phosphate in an available form.

TABLE 7

Total phosphate in available form in the surface layers (0–9 inches)						
Plot	4	5	6	7	8	9
Total phosphate ppm P_2O_5	1161	910	621	994	1043	1102
Available phosphate ppm P_2O_5. . .	5.4	8.7	1.4	3.5	9.7	4.8
% Total phosphate available. . . .	0.465	0.956	0.225	0.352	0.930	0.435
Relative availability (plot 6 = 100).	206	424	100	156	413	193

The increased proportion of total phosphorus in available form on plot 5 may have been due to the application of superphosphate over the experimental period of 58 years. Superphosphate containing 16 to 18 per cent water-soluble P_2O_5 appears to have afforded a more ample supply of readily available phosphate than the basic slag treatments with the exception of the basic slag and lime dressing given to plot 8. Plot 5 differed from other plots in both pH and available calcium having the lowest mean pH value of 6.0 and the lowest mean available-calcium content of 3000 ppm. As both these factors might be considered favourable to phosphate remaining in available form their combined influence may have been responsible for the increased availability of phosphate on this plot.

Results from the original experiment, expressed in terms of liveweight gain of sheep [5], showed that returns from the superphosphate dressing were inferior to those from the basic slag treatments. Botanical analysis of the sward [12] also demonstrated that the improvement due to superphosphate was not as evident as that from basic slag, notably in the incidence of wild white clover (*Trifolium repens* L.). It was therefore confusing that soil from plot 5 should have relatively high available-phosphate content.

The failure of superphosphate to give production comparable with that of the basic slag treatments was repeated when cereal crops were grown. The yields on plot 5 (Table 8) were below those of all the plots receiving basic slag in spite of the relatively high phosphate availability. This was interesting as the yield data from

TABLE 8

Grain yields of cereal crops (cwt./acre). Tree Field 1956 and 1957												
Original plot	4		5		6		7		8		9	
fertiliser added	Oats 1956	Wheat 1957	Oats 1956	Wheat 1957	Oats 1956	Wheat 1957	Oats 1956	Wheat 1957	Oats 1956	Wheat 1957	Oats 1956	Wheat 1957
Nil	29.4	22.1	22.9	19.4	5.9	3.5	27.3	23.2	32.5	26.5	28.4	21.5
N	33.9	25.4	31.8	23.4	10.6	5.0	33.7	26.5	35.8	25.8	27.8	25.5
P	31.5	24.0	25.9	20.4	11.8	11.9	25.0	22.5	28.8	25.0	30.9	23.7
K	26.6	22.9	24.2	20.9	9.8	6.4	26.2	22.4	29.3	26.3	28.0	22.4
Ca	28.5	24.5	23.8	20.0	11.2	6.3	23.9	21.5	29.7	21.7	31.7	22.0
NPKCa	38.8	26.7	32.8	24.5	21.6	18.5	32.9	25.0	33.0	24.7	32.4	26.6

* For rates of application see Table 2.

plot 6 suggested that phosphate was the limiting factor to optimum growth and yield of the cereal crops. The cropping potential or residual value of the original superphosphate dressings as measured by the growth and yields of the cereal crops was lower than that of the basic slag treatments.

Soil from plot 8, which had received basic slag and lime, also showed a relatively high level of available phosphate but contrasted with plot 5 in having a high pH and a high available-calcium content. This was unusual as these conditions would apparently favour the fixation of phosphorus. However, physical analyses showed that differences existed between plot 8 and the remaining plots which might have contributed towards phosphate remaining in available forms. The soil structure following the repeated basic slag and lime dressings was superior to that of other plots. The physical nature of the soil on the experiment has been reported elsewhere [3] but the differences in dispersion ratios (Table 9) typifies the results and is accepted as a reliable guide to structural stability [7 11 14]. The physical analyses of soil from plot 8 showed that its physical condition was very favourable to plant growth.

The organic matter in the soil of plot 8 was completely decomposed and thoroughly incorporated into the surface soil contrasting with plots 5 and 6 where the organic matter was only partially decomposed and was accumulating on the soil surface. This indication of an active microbial population on plot 8 agrees with Thomas and Elliot [23] who found that the nitrification and nitrogen fixing capacities of plot 8 were high compared to other

TABLE 9

Dispersion ratios. 0 to 9-inches samples					
Plot	1955 samples	1956 samples	1957 samples	1958 samples	Mean
4	10.2	12.7	14.3	14.3	12.9
5	13.1	14.6	15.8	17.6	15.3
6	14.5	16.4	19.1	17.8	16.9
7	11.3	12.2	12.5	11.5	11.9
8	8.8	9.7	10.4	10.4	9.8
9	13.4	14.2	13.6	15.2	14.1
Mean	11.9	13.3	14.3	14.5	13.5

plots. Bradley and Sicling [2] and others [8], point out that decomposition of organic matter prevents phosphate fixation over wide pH ranges and this may have reduced phosphate fixation on plot 8 under conditions which otherwise would have favoured rapid fixation.

It is notable that cereal yields on plot 8 were higher than on plot 5 in spite of the similar levels of available phosphate present in the soil. The outstanding difference lay in the physical condition of the two plots. Basic slag and lime in promoting a soil structure which favoured the growth of plants had a residual value which was superior to that of the superphosphate dressings. This was particularly evident in the second year of arable cropping when the application of 1 cwt. of triple-superphosphate failed to increase grain yield on plot 8. It has been stated that any attempt to build up reserves of phosphate in soil is fundamentally unsound and uneconomic on the acid glacial-drift soils commonly found in Great Britain [22]. However, the failure of triple-superphosphate to give increased grain yield when applied in successive years to cereals suggests that the oats and wheat were utilising available phosphate which had accumulated from the basic slag and lime dressings. If this greater cropping potential is a consistent effect of applying phosphatic fertilisers, particularly basic slag, and lime to grassland it could be an important means of further increasing the value of the long-term ley in a rotation.

SUMMARY

The distribution and condition of soil phosphate under old permanent pasture on Tree Field at Cockle Park was investigated. Two cereal crops

were then grown to estimate the residual value of the phosphatic dressings which had been applied over a long period of time.

The total-phosphate content of Tree Field soil was found to be concentrated in the surface layers and to a lesser extent at depths of 36 inches. The total-phosphate contents of horizons between depths of 12 to 24 inches were relatively low. The penetration of the phosphatic fertilisers was found to be limited to the surface horizons.

The availability of the phosphatic reserves differed in the surface layers of the plots. Plots which had received superphosphate or basic slag and lime had relatively large amounts of total phosphate in available form at these depths. Evidence of the possible utilisation of available phosphate by the original sward at a depth of 36 inches was found.

The cropping potential of the plot which had received basic slag and lime was found to be superior to that of the remaining plots.

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REFERENCES

1 Bradfield, R., The physical chemistry of soil-plant relationships. Soil Sci. Soc. Am. Proc. **11**, 3–8 (1946).
2 Bradley, D. B. and Sieling, D. H., Effect of organic substances on phosphate precipitation by iron and aluminium as influenced by pH. Soil Sci. **76**, 175–182 (1953)
3 Brockman, J. S., Ph. D. Thesis. University of Durham (1958).
4 Caldwell, A. C., Hustrulid, A., and Macgregor, J. M., The uptake and recovery of phosphorus applied broadcast to established stands of alfalfa. Unpublished data. Minnesota Agr. Exp. Sta. (1951).
5 Guides Exp. Cockle Park, 1897–1954.
6 Crowther, E. M., Subsoil structure and crop nutrition. Trans. Intern. Congr. Soil Sci. 3rd Congr. **3**, 126–139 (1935).
7 Currie, J., Ph. D. Thesis. University of Durham (1955).
8 Dalton, J. D., Russell, G. C., and Sieling, D. H., Effect of organic matter on phosphate availability. Soil Sci. **73**, 173–181 (1952).
9 Doak, B. W., Top dressing permanent pastures. New Zealand J. Sci. Technol. **24**, 141–144 (1942).
10 Glantworth, R., Properties of gleyed soil horizons. Nature **159**, 441–442 (1947).
11 Henin, S., and Turc, L., Stability of soil under various cultivations. Soils and Fertilizers Commonwealth Bur. Soil Sci. **13**, 102–108 (1950).
12 Heslop-Harrison, H., Private communication (1957)
13 Lawton, K., Robertson, L. S., Cook, R. L., and Rood, P. J., A study of the

correlation between rapid soil tests and response of legume hay to phosphorus and potassium fertilisation on some Michigan soils. Soil Sci. Soc. Am. Proc. **12**, 353–358 (1947).

14 Middleton, H. E., The properties of some soils in North Carolina. U.S. Dept. Agr. Tech. Bull. **178**, 16–21 (1930).

15 Muir, A., The condition of phosphate in gleyed horizons. Thorpes Dictionary of Applied Chemistry **8**, 151–153 (1940).

16 Nelson, L. B., Meldrum, H. R., and Pierre, W. H., Fertiliser needs of legume hay. Proc. Natl. Joint Comm. Fertilizer Application, **22**nd Ann. Meeting 20–23 (1946).

17 Rice Williams, Soil fertility data for a fifteen-year experiment on hay land. Welsh J. Agr. **16**, 132–144 (1940).

18 Roy, B. B., Ph. D. Thesis. University of Durham (1948).

19 Roy, B. B. and Thomas, B., The phosphate reserves in some old permanent pasture plots. Emp. J. Exp. Agr. **19**, 175–184 (1951).

20 Stanford, G., McAuliffe, C., and Bradfield, R., The efficiency of superphosphate top-dressed on established meadows. Agron. J. **42**, 423–426 (1950).

21 Stanford, G., and Pierre, W. H., Soil management practices in relation to phosphorus availability and use. Agronomy **4**, 243–280 (1953).

22 Stewart, A. B., Phosphate usage in Great Britain. Agronomy **4**, 427–448. (1953).

23 Thomas, B. and Elliott, F. J., Investigations on old permanent pasture. J. Soc. Chem. Ind. London **51**, 332–336 (1932).

24 Weaver, J. E. and Darland, R. W., Soil-root relationships of certain native grasses in various soil types. Ecol. Monographs **19**, 303–338 (1949).

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STUDIES ON THE RHIZOSPHERE MICROFLORA OF YELLOW BIRCH SEEDLINGS *

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INTRODUCTION

As revealed by the reviews of Clark [4], Katznelson *et al.* [13], and Starkey [26] a good deal of information exists concerning the rhizosphere population of different cultivated plants. In spite of these many observations only a few workers have paid attention to the microflora of roots of larger plants such as forest trees growing in forest soil. Harper [10] comparing banana varieties found that plants susceptible to Panama disease had a higher number of rhizosphere microflora than nonsusceptible varieties. Rombouts [22] reported that the banana rhizosphere favoured the development of micro-organisms but found little difference between susceptible and nonsusceptible varieties to Panama disease. Harley and Waid [9] showed that high light intensity favoured the colonization of the fungus *Trichoderma viride* on surfaces of beech roots. Low light intensity supported growth of *Rhizoctonia*. Koslova [14] observed that the type of bacterial microflora of the oak rhizosphere differed from that of the soil, whereas the fungal microflora was the same. Netti [20] found numbers of denitrifying bacteria in the oak rhizosphere to be 10 to 100 times greater than the number in soil not containing oak roots. According to Formisano[7], *Azotobacter* and actinomycetes in the rhizosphere of oranges, walnuts, and olives reached their greatest numbers during the flowering stage; for corn, potatoes, and beans, numerical peaks were obtained during the period of physiological maturation. Gyllenberg and Hanioja [8] recently reported that the rhi-

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zosphere effect of graminaceous plants was higher than that of trees and shrubs. The present paper deals with studies on the rhizosphere microflora of yellow birch (*Betula lutea* Michx. f.). This investigation was undertaken in cooperation with Dr. F. L. Raymond of the Forest Biology Laboratory, Maple, Ontario, who has conducted exploratory studies on microflora of yellow birch seedlings.

EXPERIMENTAL METHODS

The dilution-tube technique and plate counts were employed to determine numbers of certain groups of micro-organisms in the rhizosphere of yellow birch, and in soil a short distance away from the roots. Samples for analysis were obtained as described by Lochhead [15] and suitable dilutions were prepared. One-milliliter aliquots of each dilution were added in quintuplicate to tubes of appropriate media which were incubated at 26°C and examined at regular intervals. Ammonifying bacteria were determined as described previously [11]. Total numbers of anaerobic, anaerobic gas- and anaerobic acid-producing bacteria were determined by growing in deep peptone-glucose agar tubes [6] containing bromocresol purple at a final concentration of 0.0008 per cent. For methylene-blue-reducing bacteria the method of Katznelson and Rouatt [12] was used. Bacteria and actinomycetes were enumerated on soil-extract agar, and numbers of fungi were obtained by plating on rose bengal-streptomycin agar [18]. Nutritional grouping of bacteria isolated from representative plates was carried out using the technique of Lochhead and Chase [16] as later modified [27].

Total N of the soil was determined by the Kjeldahl method. Soil organic matter was determined as described by Peech *et al.* [21].

For the particle-size distribution of soil the pipette method [1] was used. The pH of the soil was determined with a glass electrode.

In the initial experiments it seemed desirable to investigate the rhizosphere effect of yellow birch under natural conditions. The location selected was situated approximately 20 miles west of Ottawa. The tree cover consisted of a coniferous-deciduous mixture, of which yellow birch comprised about 5 per cent of the stand. This stand was on a well drained sand of podzol nature. In July, 1957, yellow birch trees, 10 to 20 years old, were chosen and samples of soil and rhizosphere were taken from the humus layer.

The results showed a slight or no rhizosphere effect for the majority of microbial groups listed above. However, in a few cases, bacteria for example, a rhizosphere:control (R:C) ratio of 8 to 10 was obtained. This failure to obtain a pronounced rhizosphere effect was thought to be associated with the nature of the control soil sample. The fact that the surface of the forest soil was covered by a dense growth of smaller plants made the control soil of doubtful usefulness because root debris and rhizosphere organisms of other plants probably were incorporated with the soil in sampling.

In order to eliminate the rhizosphere effect of other plants a small forest

soil plot was established in the greenhouse. In this study the A, B, and C horizons of a forest soil were placed in a large, rectangular concrete tank the interior dimensions of which were 144 in. long, 18 in. wide and 18 in. high. To remove most of the plant debris and to overcome the heterogeneous condition of the soil, each soil horizon was screened through a 2-mm sieve and well mixed.

In October of 1957 three-year old yellow birch seedlings were planted about 8 in. apart in the upper humus layer. At planting time the seedlings were dormant. To promote rapid growth the chamber was lighted from 6 a.m. to 12 p.m. with daylight-type neon bulbs. Measurement of light radiation emitted by the bulbs showed that the crowns of the seedlings were receiving 300 foot-candles of light. The plants also received sunlight but no attempt was made to measure its intensity. When the seedlings broke dormancy on January 3, 1958, a small section of the plot was walled off and the light intensity for this area was reduced to 25 foot-candles. No sunlight was allowed to enter this latter section. This part of the experiment was conducted to determine whether or not light intensity affects the rhizosphere population. Samples of the humus layer and of roots were taken periodically, before and after the plants broke dormancy.

RESULTS

Results of chemical and physical analysis of the forest soil used in the greenhouse experiment are presented in Table 1. The pH

TABLE 1

Chemical and physical characteristics of forest soil used in greenhouse							
Soil Horizon	Depth (inches)	pH	Total N (%)	Organic Matter (%)	Particle size distribution		
					Sand > 0.05 mm (%)	Silt 0.05–0.002 mm (%)	Clay <0.002 mm (%)
A	0–6	4.3	0.79	17.1	59	22	19
B	6–13	5.6	0.12	2.3	65	28	7
C	13–18	6.0	0.06	1.5	69	26	5

values indicate that the A horizon was quite acid; the B and C horizons were less acid. As was to be expected total nitrogen and quantity of organic matter were higher in the A horizon than in the B and C horizons. The use of a soil-textural diagram [28] revealed that the texture of the three horizons was sandy loam.

Effect of plant growth on micro-organisms of the rhizosphere

The numbers of different microbial groups at different stages of plant growth are shown in Table 2. The figures presented concern

TABLE 2

The incidence of certain groups of soil micro-organisms on the roots of yellow birch seedlings, at different stages of growth *

Group	Weekly growth periods, before and after seedlings broke dormancy														
	4 weeks before			0 weeks			3 weeks after			7 weeks after			28 weeks after		
	C **	R †	R:C ‡	C	R	R:C	C	R	R:C	C	R	R:C	C	R	R:C
Plate count															
Bacteria.	11.8	125.3	10.6	18.1	94.1	5.2	20.5	80.9	3.9	25.0	251.0	10.0	23.4	353.6	15.1
Actinomycetes. .	9.3	47.4	5.1	5.2	14.7	2.8	7.2	14.2	2.0	2.8	13.6	4.9	2.5	13.0	5.2
Fungi.	0.5	1.2	2.4	0.5	1.1	2.2	0.6	1.2	2.0	1.2	1.9	1.6	0.8	1.0	1.3
Physiological															
Ammonifying . .	61.7	1,081.2	17.5	27.0	93.8	3.5	40.5	122.8	3.0	45.5	1,576.0	34.6	33.9	1,315.5	38.8
Reducing methylene blue .	13.1	78.2	6.0	9.5	12.5	1.3	9.7	21.9	2.3	15.6	47.7	3.1	3.8	171.3	45.1
Anaerobic bacteria	N.D. §	N.D.	N.D.	6.7	13.1	2.0	1.2	13.9	11.6	1.5	1.5	1.0	1.7	2.5	1.5
Anaerobic acid producers. . . .	2.7	1.2	N.R. §§	2.1	4.7	2.2	6.1	4.4	N.R.	0.7	0.8	1.1	1.7	2.8	1.6
Anaerobic gas producers. . . .	0.010	0.055	5.5	0.020	0.010	N.R.	0.010	0.020	2.0	0.002	0.004	2.0	0.012	0.060	5.0

* Numbers expressed in millions per gram oven-dry soil.

** C = Control soil.

† R = Rhizosphere soil.

‡ $R:C = \frac{\text{Numbers in rhizosphere soil.}}{\text{Numbers in control soil.}}$

§ N.D. = Numbers not determined.

§§ N.R. = No rhizosphere effect.

only those groups of micro-organisms that showed a rhizosphere effect, however slight. Although aerobic and anaerobic cellulose-decomposing bacteria, aerobic spore-forming bacteria, and algae were also counted during the course of the investigation, the data have been omitted because numbers were low and of questionable significance.

In general, a comparison of these results (Table 2) with those previously obtained in this laboratory [11 17] shows that the R : C ratios of yellow-birch roots are lower than those of cropped plants. Nevertheless a small rhizosphere effect may be observed. Of the micro-organisms studied, bacteria, ammonifying forms and methylene-blue reducers appear to be the predominant groups in the rhizosphere of yellow birch. Actinomycetes also showed a rhizosphere effect; the remaining groups were affected only very slightly. The numbers of bacteria, ammonifying and methylene-blue-reducing organisms decreased in the rhizosphere after the plants broke dormancy, and then increased in the later periods of growth.

Cultures were isolated from the soil-extract agar plates used for bacterial counts and inoculated into several different media in order to classify them according to their nutritional needs. These results are presented in Table 3. There is no appreciable difference between the rhizosphere and the control soil population. This is

TABLE 3

Nutritional classification of bacteria on the roots of yellow birch seedlings*

Group	Weekly growth periods, after seedlings broke dormancy			
	0 weeks		7 weeks after	
	Control	Rhizosphere	Control	Rhizosphere
B	0 **	4	0	0
A	68	61	51	60
G	4	8	0	0
Y	28	20	40	38
YS	0	7	9	2

* Approximately 100 isolates were tested in each case.

** Per cent of total organisms.

of interest because studies in this laboratory [17] with a wide variety of cropped plants have shown that, on the average, there are about 3 times as many amino-acid-requiring bacteria in the root zone

as in the soil more distant from the plant. It was also found [17] that bacteria with requirements for the complex factors in yeast and soil extracts were relatively more numerous in the soil. However, since no rhizosphere effect could be obtained with the nutritional groups the results tend to confirm the findings that the microflora of yellow-birch roots growing in the humus layer is not markedly different from that of the adjacent soil (Table 2).

After 28 weeks growth roots of the seedlings grown under high light intensity had penetrated and branched throughout the B horizon. Therefore, additional experiments were conducted to compare the rhizosphere effect in both the humus and the lower mineral soil layer. The results of these experiments are presented in Table 4. In the case of total bacterial numbers, and ammonifying

TABLE 4

The incidence of certain groups of soil micro-organisms on the roots of yellow birch seedlings growing in two soil horizons*

Groups	A horizon			B horizon		
	C	R	R:C	C	R	R:C
Plate count						
Bacteria	23.4	353.6	15.1	3.1	179.1	57.8
Actinomycetes	2.5	13.0	5.2	3.3	15.7	4.8
Fungi	0.8	1.0	1.3	0.07	0.1	1.4
Physiological						
Ammonifying	33.9	1,315.5	38.8	2.8	392.0	140.0
Reducing methylene blue	3.8	171.3	45.1	1.0	418.9	418.9
Anaerobic bacteria	1.7	2.5	1.5	1.2	3.1	2.6
Anaerobic acid producers	1.7	2.8	1.6	0.2	0.4	2.0
Anaerobic gas producers	0.012	0.060	5.0	0.002	0.005	2.5

* See footnotes Table 2.

and methylene-blue-reducing bacteria a greater R : C ratio was obtained in the B horizon than in the A horizon, but with actinomycetes, fungi, anaerobic bacteria, anaerobic acid- and anaerobic gas-producing bacteria no difference was observed.

Table 5 shows the results of the nutritional and physiological classification of the bacteria isolated from the above samples. The root zone within the B horizon contains a higher percentage of amino-acid-requiring bacteria and a lower relative number of those requiring the more complex factors of yeast and soil extracts. The ammonifying group also appears to be stimulated somewhat

TABLE 5

Nutritional and physiological groups of bacteria on the roots of yellow birch seedlings growing in two soil horizons *

Group	A horizon		B horizon	
	Control	Rhizosphere	Control	Rhizosphere
Nutritional				
B	0	1	2	0
A	61	62	37	53
G	0	0	2	3
Y	25	21	43	35
YS	14	16	16	9
Physiological				
Ammonifying	86	89	72	86
Reducing methylene blue	55	48	46	50

* See footnotes Table 3.

in the rhizosphere of the B horizon. On the other hand the A horizon does not show any of these effects. These observations, along with those shown in Tables 2 and 3, suggest that certain soils, particularly those with a high organic-matter content, may mask the rhizosphere effect.

Effect of light on the rhizosphere microflora of yellow birch

In this experiment the soil and rhizosphere of the humus layer were examined 16 weeks after the plants had broken dormancy. During this period the plants under high light intensity had grown about 15 inches, whereas those under low light had grown about 3 inches. Although no staining procedure was used, visual and microscopical examination revealed that under high light intensity, the roots had structures which appeared to be mycorrhizal clusters; such clusters were virtually absent where the light intensity was low. These observations are in agreement with the findings of Bjorkman [2]. Determinations of the numbers of different microbial groups and of the incidence of nutritional and physiological types were also made. However, as the results were inconclusive they will not be presented at this time.

DISCUSSION

In the foregoing experiments efforts were made to determine the incidence of certain groups of soil micro-organisms in the rhi-

zosphere soil of yellow birch seedlings as influenced by age of plant, type of soil horizon, and light intensity.

Periodic testing of the A horizon for 28 weeks showed that only the bacterial count and numbers of ammonifying and methylene-blue-reducing bacteria were appreciably affected by plant growth. In the root zone these organisms declined in numbers when the seedlings broke dormancy. Later on their numbers increased. This agrees with the conclusions of Katznelson [11], Starkey [25], and others [4 26] that the rhizosphere effect of a number of cultivated plants was more pronounced in the stage of greatest vegetative development. Throughout the experiment the methylene-blue reducers, ammonifying organisms and bacteria in general were the most abundant and showed the greatest rhizosphere effect. Other groups such as actinomycetes, fungi and the anaerobic gas- and acid-producers were less affected by rhizosphere conditions, as had been shown also by other workers [11 26].

Although a rhizosphere effect was obtained with certain groups of organisms, yellow birch seedlings grown in the A horizon did not appear to exert a marked influence upon the soil population. Nutritional and physiological classification of bacterial isolates from the same soil also showed that the rhizosphere microflora was not too much different from that of the soil. In contrast to these findings a pronounced rhizosphere effect was observed in the lower B horizon. Here marked differences were obtained with ammonifying, methylene-blue-reducing, and total numbers of bacteria. Further tests with bacterial isolates also showed that the B horizon affected the percentage distribution of the nutritional and physiological groups and, especially those organisms which require amino acids for optimum growth.

Although not in full agreement, the results are somewhat similar to those of Gyllenberg and Hanioja [8] who observed that "the roots of trees and shrubs exert a negative rhizosphere effect in A_0 horizon. In the deeper B horizon, on the other hand, distinct promoting effect was found with the seedlings of birch and spruce". They have suggested that the effects noted may be ascribed to two factors, (a) the soil conditions, and (b) the woody parts of the roots of trees and shrubs have an inhibiting effect upon soil bacteria. It would appear therefore that soils high in organic matter content tend to mask the rhizosphere effect of plant roots.

These findings are supported by the work of Clark [3] and Clark and Thom [5] who added organic manures to wheat and cotton and found that the characteristic root-surface microflora was scarcely affected. Presumably the organic manuring decreased the difference between the rhizosphere and control soils by permitting a greater development of bacteria in the latter. Similar results have been found with regard to effect of organic manures on the balance between the various nutritional groups of soil bacteria [23].

The most marked effect of low light intensity was a severe stunting of the plants and the absence of mycorrhizal clusters on the roots. Microbial analyses of these roots as compared with those of the plants grown under high light intensity yielded results which were difficult to interpret and which cannot, therefore, be discussed at this time. It is conceivable that the effect of light on the rhizosphere microflora would be obscured and complicated by the high organic-matter content of the soil and by the presence of mycorrhiza. In fact, as mentioned by Slankis [24], two Russian workers [19 29] have reported "that the population of fungi and bacteria was much higher in the rhizosphere of tree seedlings with mycorrhiza". Consequently it is planned to study this effect in a soil of low organic-matter content in the presence and absence of such fungi.

SUMMARY

The rhizosphere microflora of yellow birch seedlings grown in a forest soil in tanks in the greenhouse has been investigated. In general it was found that the rhizosphere effect of yellow birch roots in the A horizon was lower than that observed with cropped plants; nevertheless a stimulation of microbial development was obtained. Of the micro-organisms studied, bacteria, and ammonifying and methylene-blue-reducing organisms appeared to be predominant types in the root zone. Counts were, on the whole, lowest when plants broke dormancy and began to increase in the later periods of growth. There was no appreciable difference between control and rhizosphere soils in regard to incidence of specific nutritional types of bacteria. Analysis of the rhizosphere population of roots growing in the B horizon showed a distinctly greater rhizosphere effect than that obtained in the A horizon. The data suggest that the rhizosphere effect in the latter was obscured because of its high organic-matter content and its abundant and active microflora.

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REFERENCES

1 Baver, L. D., Soil Physics. Third Ed. John Wiley and Sons, Inc., New York (1956).
2 Bjorkman, E., The ecological significance of the ectotrophic mycorrhizal association in forest trees. Svensk Botan. Tidskr. **43**, 233–262 (1949).
3 Clark, F. E., Effects of soil amendments upon the bacterial populations associated with roots of wheat. Trans. Kansas Acad. Sci. **42**, 91–96 (1939).
4 Clark, F. E., Soil microorganisms and plant roots. Advances in Agron. **1**, 241–288 (1949).
5 Clark, F. E. and Thom, C., Effects of organic amendments upon the microflora of the rhizosphere of cotton and wheat. Trans. Third Comm. Intern. Soc. Soil Sci. New Brunswick, N.J. **A**, 94–100 (1939).
6 Fred, E. B. and Waksman, S. A., Laboratory Manual of General Microbiology. McGraw-Hill Book Company, Inc., New York (1928).
7 Formisano, M., Ricerche microbiologiche sulla rizosfera delle piante coltivate nei terreni della Campania. Ann. Fac. Agrar. R. Univ. Napoli **21**, Serie III, 1–95 (1955).
8 Gyllenberg, H., and Hanioja, P., The "rhizosphere effect" of graminaceous plants in virgin soils. III. Comparison with the effects of other plants. Physiol. Plantarum **9**, 441–445 (1956).
9 Harley, J. L., and Waid, J. S., The effect of light upon the roots of beech and its surface population. Plant and Soil **7**, 96–112 (1955).
10 Harper, J. L., Studies in the resistance of certain varieties of banana to Panama disease. Part II. The rhizosphere. Plant and Soil **2**, 383–394 (1950).
11 Katznelson, H., The "rhizosphere effect" of mangels on certain groups of soil microorganisms. Soil Sci. **62**, 343–354 (1946).
12 Katznelson, H. and Rouatt, J. W., Studies on the incidence of certain physiological groups of bacteria in the rhizosphere. Can. J. Microbiol. **3**, 265–269 (1957).
13 Katznelson, H., Lochhead, A. G., and Timonin, M. I., Soil microorganisms and the rhizosphere. Botan. Rev. **14**, 543–587 (1948).
14 Koslova, E. I., A study of the generic and specific composition of the microflora of the oak rhizosphere. Mikrobiologiya **24**, 558–564 (1955). (Soils and Fertilizers, Commonwealth Bur. Soil Sci. **19**, 58 (1956)).
15 Lochhead, A. G., Qualitative studies of soil microorganisms. III. Influence of plant growth on the character of the bacterial flora. Can. J. Research **C 18**, 42–53 (1940).
16 Lochhead, A. G. and Chase, F. E., Qualitative studies of soil microorganisms. V. Nutritional requirements of the predominant bacterial flora. Soil Sci. **55**, 185–195 (1943).
17 Lochhead, A. G. and Rouatt, J. W., The "rhizosphere effect" on the nutritional groups of soil bacteria. Soil Sci. Soc. Am. Proc. **19**, 48–49 (1955).
18 Martin, J. P., Use of acid, rose bengal, and streptomycin in the plate method for estimating soil fungi. Soil Sci. **69**, 215–232 (1950).
19 Misustin, E. N., Die Mikroflora des Bodens und die Mykorrhiza-Bildung bei der Eiche. Agrobiologya **2**, 27–39 (1951). (Cited by Slankis, ref. 24).

20 Netti, I. T., Denitrifying bacteria of the oak rhizosphere. Mikrobiologiya **24**, 429–434 (1955). (Soils and Fertilizers, Commonwealth Bur. Soil Sci. **19**, 60 (1956)).
21 Peech, M., Alexander, L. T., Dean, L. A., and Reed, J. F., Methods of soil analysis for soil-fertility investigations. U.S. Dept. Agr. Circ. **757**, 5–7 (1947).
22 Rombouts, J. E., The microorganisms in the rhizosphere of banana plants in relation to susceptibility or resistance to Panama disease. Plant and Soil **4**, 276–288 (1953).
23 Rouatt, J. W., and Lochhead, A. G., Qualitative studies of soil microorganisms. XIII. Effect of decomposition of various crop plants on the nutritional groups of soil bacteria. Soil Sci. **80**, 147–154 (1955).
24 Slankis, V., The role of auxin and other exudates in mycorrhizal symbiosis of forest trees. *In* The Physiology of Forest Trees. Edited by K. V. Thimann. Ronald Press Co., New York, (1958), pp. 427–443.
25 Starkey, R. L., Some influence of the development of higher plants upon the microorganisms of the soil. II. Influence of the stage of plant growth upon the abundance of organisms. Soil Sci. **27**, 355–378 (1929).
26 Starkey, R. L., Interrelations between microorganisms and plant roots in the rhizosphere. Bacteriol. Rev. **22**, 154–172 (1958).
27 Stevenson, I. L. and Rouatt, J. W., Qualitative studies of soil microorganisms. XI. Further observations on the nutritional classification of bacteria. Can. J. Botany **31**, 438–447 (1953).
28 Toogood, J. A., A simplified textural classification diagram. Can. J. Soil Sci. **38**, 54–55 (1958).
29 Tribunskaja, A., Untersuchungen der Rhizospharenmikroflora der Kiefernsamlinge. Mikrobiologya **24**, 188–192 (1955). (Cited by Slankis, ref. 24).

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SOME ENVIRONMENTAL FACTORS INFLUENCING RADIOSTRONTIUM UPTAKE BY PLANTS *

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INTRODUCTION

Radiostrontium is foremost among the by-products of nuclear fission presently considered to be hazardous to man because it may be transmitted readily through the food chains leading to man [2 5 8 16]. The main source of radiostrontium contamination of the natural environment has been from the fallout debris of nuclear detonations. It is probable that radiostrontium may continue to be dispersed into the natural environment from other sources upon future utilization of nuclear energy. The continued production of radiostrontium has motivated studies of various factors that may inhibit the transfer of strontium through the soil-to-plant phase of the food chain leading to man. Data reported in this paper show some plant species effects and the influence of short-term variations in root temperature, light intensity and photoperiod on Sr^{90} uptake by crop plants.

MATERIALS AND METHODS

Several different species of crop plants were grown on flats containing 40 kg of Vina loam to which Sr^{90} in secular equilibrium with Y^{90} had been applied at an activity level of 4,120 dis./sec/g dry soil. White Rose potato was grown on 20 kg of Sassafras sandy loam, Hanford sandy loam, and Sorrento loam treated with Sr^{90} at an activity level of 128 dis./sec/g dry

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soil. Detailed properties of these soils and the methods of applying Sr^{90} to them have been reported [13]. Crop age and some identifying soil properties are shown in Table 1 and 2.

Bean and barley plants were grown on nutrient solution under light intensity of 1,000 foot-candles supplied for 16 hours of every 24-hour cycle from fluorescent tubes supplemented by Mazda lamps. Root temperature was maintained at 27° ± 1°C by placing culture jars in controlled water baths. The temperature of air filtered through activated-carbon filters varied from 27° to 30°C. Fifty barley plants and 2 bean plants per culture were grown on 3 liters of balanced nutrient solution (Ca, K and Mg = 8.33 me/l) until the environmental variables were introduced. The solutions were renewed every 6 days and continuously aerated. The environmental variables were introduced when the bean plants were 24 days old and the barley plants were 18 days old. Five replicates of each treatment were maintained. After a 24-hour stabilization period to enable the plants to adjust to the environmental changes, Sr^{90} was applied to the nutrient solution at an activity level of 5 μc Sr^{90} per liter; stable strontium was applied at the level of 1.0 me Sr per liter.

The plants were harvested after a 24-hour period for Sr^{90} absorption. This short exposure period was used to reduce the influence of altered growth rates caused by the environmental changes. One-half gram samples of dry plant tissue were radioassayed for Sr^{90} in equilibrium with Y^{90} using an end-window GM detector (1.9 mgm/cm^2). Appropriate counting corrections were applied.

RESULTS

Effect of plant species

Table 1 shows the Sr^{90} content of several different plant species grown on Vina loam. Millet obtained from two to three times more Sr^{90} than was taken up from the soil by barley, oats or wheat. For each of the grain crops, the concentration of Sr^{90} in the grain was only about one-fifth of the Sr^{90} content in the forage. The highest concentration of Sr^{90} was found in leaves of young turnip plants and swiss chard. The relative decreasing order of Sr^{90} uptake per unit dry weight of mature plant tops was turnip = millet > swiss chard > ladino clover > broccoli > soybeans > barley > oats > wheat > spinach. In a previous study with other crop plants, Romney *et al.* [13] found that the relative decreasing order of Sr^{90} uptake was radish > bean > carrot > lettuce > barley.

Very low levels of Sr^{90} were found in White Rose potato tubers in comparison to high levels found in the plant tops (Table 2). Soil

TABLE 1

Sr^{90} uptake from Vina loam * by different plant species

Plant species	Crop age, days	Sr^{90} content, dis./sec/g
Cereals		
Millet, young forage	74	3,756.0
Millet, milk-stage grain	74	554.2
Millet, mature forage	95	6,659.4
Millet, dough-stage grain	95	996.7
Oats, young forage	41	1,784.4
Oats, mature leaves	182	2,142.7
Oats, ripe grain	182	1,004.4
Barley, young forage	41	2,501.0
Barley, mature forage	150	2,269.6
Barley, ripe grain	150	667.3
Wheat, young forage	41	2,082.7
Wheat, mature forage	81	1,829.7
Wheat, dough-stage grain	81	534.0
Vegetables		
Spinach	48	1,560.3
Swiss chard	72	6,109.4
Turnip, young plant	52	9,152.9
Turnip, greens	70	9,892.2
Turnip, immature root	70	4,392.9
Turnip, mature leaves	92	6,581.1
Turnip, mature root	92	3,843.0
Broccoli, edible stage	120	3,129.3
Broccoli, mature tops	145	2,485.6
Legumes		
Ladino clover, 1st harvest	108	4,872.3
Ladino clover, 2nd harvest	140	4,523.2
Soy bean, forage	62	2,685.5

* Sr^{90} activity level of 4,120 dis./sec/g soil; soil pH 6.5; cation exchange capacity 14.25 me/100 g; exchangeable strontium 0.047 me/100 g; exchangeable calcium 8.20 me/100 g.

type influenced Sr^{90} uptake by potato. As was previously observed for other crop plants [13], the Sr^{90} uptake was highest from the acidic Sassafras soil which was low in calcium supply and the uptake was lowest from the Sorrento soil high in calcium supply. The inhibiting influence of calcium on strontium is attributable to

TABLE 2

Sr^{90} uptake from Sassafras, Hanford, and Sorrento soils * by potato		
Soil properties	Plant part	Sr^{90} content † dis./sec/g
Sassafras – pH 4.6		
(Cation exchange capacity 6.03 me/100 g,	Tops	5,612
exchangeable strontium 0.002 me/100 g,	Tuber skin	40
exchangeable calcium 0.40 me/100 g)	Peeled tuber	95
Hanford – pH 6.6		
(Cation exchange capacity 8.22 me/100 g,	Tops	3,136
exchangeable strontium 0.032 me/100 g,	Tuber skin	19
exchangeable calcium 4.50 me/100 g)	Peeled tuber	72
Sorrento – pH 8.0		
(Cation exchange capacity 15.10 me/100 g,	Tops	1,520
exchangeable strontium 0.070 me/100 g,	Tuber skin	14
exchangeable calcium 12.44 me/100 g)	Peeled tuber	35

* Sr^{90}-activity level of 128 dis./sec/g soil.

† Mean of 3 replicates grown 112 days.

the complementary ion relationships between these two elements [1 5 10 14]. In alkaline-calcareous soils, there is also the inhibiting influence of alkaline reaction and high bicarbonate concentration on the availability of soluble strontium.

Effect of root temperature

Barley and bean plants were grown on nutrient solutions at root temperature treatments of 27°, 17°, and 7°C. Lowering the root

TABLE 3

Effect of root temperature on 24-hour absorption of Sr^{90} by barley and beans grown on nutrient solution		
Root temperature	Sr^{90} content of dry tops /dis./sec/g)	
	Barley	Bean
27°C	563	1,140
17°C	611	1,268
7°C	189	588
L.S.D. (5%)	47.3	179.2
L.S.D. (1%)	69.3	304.3
Q10 values		
(17–27°C)	—	—
(7–17°C)	3.2	2.2

temperature from 27° to 17°C caused a significant increase ($P = 0.05$) in Sr^{90} uptake by barley. At the 7°C root temperature treatment, Sr^{90} uptake was very significantly decreased ($P = 0.01$) in barley and bean plants (Table 3). The Q10 values greater than 2.0 for plants grown at 17°C and 7°C would indicate that strontium uptake is chemically controlled rather than the result of physical phenomena; Q10 values for reactions depending only upon physical phenomena usually vary from 1.0 to 1.5.

From data presented by Hylmo [7] on calcium uptake by peas during a 24-hour absorption period, a Q10 value of 2.0 was calculated for root temperature changes from 10° to 20°C. Hoagland and Broyer [6] obtained Q10 values of nearly three for potassium uptake at root temperature change between 0.5°C and 20°C.

Effect of light intensity and photoperiod

The uptake of Sr^{90} by barley and beans from nutrient solution was significantly reduced ($P = 0.01$) when the light intensity was decreased from 1,000 to 450 foot-candles by shading the plants with sheets of cheesecloth (Table 4). The effects on Sr^{90} uptake of

TABLE 4

Effect of light intensity on 24-hour absorption of Sr^{90} by barley and beans grown on nutrient solution at 27°C

Light intensity (foot-candles)	Sr^{90} content of dry tops (dis./sec/g)	
	Barley	Bean
1,000	453	1,162
450	47	167
L.S.D. (5%)	52	113
L.S.D. (1%)	117	173

varying the amount of light and darkness to which barley and bean plants were exposed during a 24-hour period are shown in Table 5. In this experiment Sr^{90} was added to the nutrient solution at the same time the photoperiod treatment was introduced. The uptake of Sr^{90} was significantly reduced at light-exposure periods of less than 12-hours duration. One-third to one-half of the total amount of Sr^{90} obtained during the 24-hour absorption period was taken up independent of exposure to light.

The influence of light on strontium uptake is probably an indirect

effect of the rise in temperature concomitant with light absorption and from photochemical reactions. A rise in temperature has been shown to cause increased transpiration rates and generally results

TABLE 5

Effect of photoperiod on 24-hour absorption of Sr^{90} by barley and beans grown on nutrient solution at 27°C

Hours of light exposure		Sr^{90} content of dry tops (dis./sec/g)	
Light	Dark	Barley	Beans
24	0	539	1,279
16	8	518	987
12	12	409	1,007
8	16	256	546
0	24	273	421
L.S.D. (5%)		105	340
L.S.D. (1%)		140	464

in increased mineral uptake. Photochemical processes are known to enhance plant uptake of inorganic salts probably by light-modified changes in permeability, viscosity, and protoplasmic streaming [3 4 17 18].

DISCUSSION

The relative degree of hazard that might arise from radiostrontium depends upon the extent to which it is transmitted through the food chains leading to man. In this respect the soil-to-plant phase of the food chain will play an important role in the event that crop-producing soils become contaminated with soluble forms of radiostrontium. Any practical means of reducing the transfer of radiostrontium from soils to plants must be considered. It has been shown that liming of soils of low calcium status will significantly reduce crop uptake of radiostrontium and that plant uptake of radiostrontium is inversely correlated with the levels of available calcium in soils [1 5 10 14]. The addition of high levels of organic matter also will inhibit radiostrontium uptake by crop plants from contaminated soils [11]. The inherent capacity of different plant species to take up more or less strontium from soils also must be considered because the difference in the amount of radiostrontium taken up by two different crop plants from the same soil may be greater than the effects produced by any practical treatment

man could apply. Certain plants or parts of plants which concentrate high levels of radiostrontium could be eliminated from the human diet, should the need arise. Fortunately, the potato tuber and the fruits and grains of most food crop plants appear to concentrate relatively low levels of radiostrontium in comparison to the foliage. Meat-supplying animals that may have to eat the plant foliage would, in turn, dilute the amount of Sr^{90} transferred to man by way of meat products.

Although it is not practical to alter soil temperature, light intensity or photoperiod over any given large agricultural area, the inhibiting effects of these environmental factors on radiostrontium uptake probably are manifest in climatic regions which experience reduced light intensity, shorter day length and cooler soil temperature.

SUMMARY

The amounts of Sr^{90} taken up from Vina soil varied among several different crop plants by a factor as high as 10 on the basis of dry weight. The relative decreasing order of Sr^{90} uptake by mature plant tops was turnip = millet > Swiss chard > Ladino clover > broccoli > soybeans > barley > oats > wheat > spinach. For each of the cereal crops, the concentration of Sr^{90} in the grain was only about one-fifth of the Sr^{90} in the forage. The tubers of White Rose potato grown on Sassafras, Hanford and Sorrento soils contained only about one-fiftieth of the Sr^{90} concentrated in the tops. The Sr^{90} uptake by potato was inversely correlated with the level of plant-available calcium in Sassafras, Hanford and Sorrento soils.

Lowering of root temperature from 17° to 7°C significantly reduced Sr^{90} uptake by barley and beans during a 24-hour absorption period. Q10 values indicated that strontium uptake was chemically controlled rather than the result of physical phenomena.

A decrease in light intensity from 1,000 to 450 foot-candle significantly reduced Sr^{90} uptake by barley and beans. The uptake of Sr^{90} also was reduced as the light exposure period was decreased. One-third to one-half of the total amount of Sr^{90} obtained during a 24-hour absorption period was taken up independent of exposure to light.

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REFERENCES

1 Bowen, H. J. M. and Dymond, J. A., Uptake of calcium and strontium by plants from soils and nutrient solutions. J. Exptl. Botany **7**, 264–272 (1956).

2 Comar, C. L., Russell, R. S., and Wasserman, R. H., Strontium-calcium movement from soil to man. Science **126**, 485-492 (1957).

3 Curtis, O. F., Leaf temperatures and the cooling of leaves by radiation. Plant Physiol. **11**, 343–364 (1936).

4 Freeland, R. O., Effect of transpiration on the absorption and distribution of mineral salts in plants. Am. J. Botany **23**, 335–362 (1936).

5 Fuller, W. H. and Flocker, W. J., The uptake of radiostrontium by certain type crops from calcareous soils. Ariz. Agr. Expt. Sta. Bull. **130** (1955).

6 Hoagland, D. R. and Broyer, T. C., Accumulation of salt and permeability in plant cells. J. Gen. Physiol. **25**, 865–880 (1942).

7 Hylmo, B., Transpiration and ion absorption. Physiol. Plantarum **6**, 333–405 (1953).

8 Jacobson, L. and Overstreet, R., The uptake by plants of plutonium and some products of nuclear fission adsorbed on soil colloids. Soil Sci. **65**, 129–134 (1948).

9 Klechkovsky, V. M., On the behavior of radioactive fission products in soil, their absorption by the plants and their accumulation in crops. Acad. Sci. U.S.S.R. (Translated from Russian by U. S. Atomic Energy Comm. Rep. AEC-TR **2867**) (1957).

10 Menzel, R. G., Competitive uptake by plants of potassium, rubidium, cesium and calcium, strontium, barium from soils. Soil Sci. **77**, 419–425 (1954).

11 Nishita, H., Kowalewsky, B. W., and Larson, K. H., Influence of soil organic matter on mineral uptake by barley seedlings. Soil Sci. **82**, 307–318 (1956).

12 Rediske, J. H. and Selders, A. A., The absorption and translocation of strontium by plants. Plant Physiol. **28**, 594–605 (1953).

13 Romney, E. M., Neel, J. W., Nishita, H., Olafson, J. H., and Larson, K. H., Plant uptake of Sr^{90}, Y^{91}, Ru^{106}, Cs^{137}, and Ce^{144} from soils. Soil Sci. **83**, 369–376 (1957).

14 Romney, E. M., Alexander, G. V., Rhoads, W. A., and Larson, K. H., Influence of calcium on plant uptake of Sr^{90} and stable strontium. Soil Sci. **87**, 160–165 (1959).

15 Russell, R. S. and Milbourn, G. M., Rate of entry of radioactive strontium into plants from soil. Nature **180**, 322–324 (1957).

16 Russell, R. S. and Ellis, F. B., The movement of strontium-90 through food chains. Soils and Fertilizers Commonwealth Bureau Soil Sci. **21**, 269–273 (1958).

17 Withrow, R. B., Light as a modifying influence on the mineral nutrition of plants. *In*: Mineral Nutrition of Plants. Univ. of Wisconsin Press, pp. 389–410 (1951).

18 Wright, K. E., Transpiration and absorption of mineral salts. Plant Physiol. **14**, 171–174 (1939).

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AN EVALUATION OF THE MAXIMUM RADIUS OF THE ROSETTE AS A GROWTH INDEX FOR SUBTERRANEAN CLOVER

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INTRODUCTION

The advantage of having a good index of yield in the early stages of growth in plant nutrition work is obvious, particularly in large factorial experiments where the randomization of replicates makes the task of observing small yield differences difficult. Anderson [2] and Millikan [4] have referred to the use of the rosette diameter to indicate the treatment differences in subterranean clover, but no attempt has been made to assess the fidelity of this measurement as a yield index. In this work, such an evaluation has been undertaken using the *maximum radius* of the rosette as the yield index. Early results showed that although leaf number was also a good index of relative dry weight, the time required for counting would be a serious disadvantage.

The value of the maximum-radius measurement for subterranean clover is due to its habit of growth. In the early vegetative stage it consists of a small, upright centre shoot surrounded radially by more or less prostrate petioles. At a later vegetative stage stem internodes elongate rapidly and with onset of inflorescence peduncles arise from the nodes and may grow very long, but still maintain a radial disposition. The rate of development through these stages depends mainly on photoperiod and temperature. This has been thoroughly discussed by Aitken [1].

The distance from the centre shoot to the base of any leaf can be rapidly measured. Measuring to the leaf that is furthest from the centre shoot gives the 'maximum radius' (Fig. 1). The correlation of this value with dry weight has been tested under varying con-

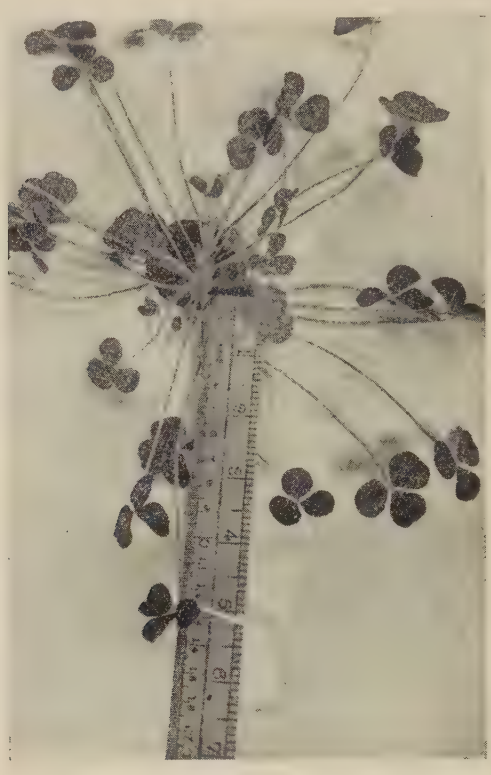

Fig. 1. Maximum radius measurement.

ditions over the last three years. Early results obtained incidentally to another study and related merely to the weight values obtained at harvest, were sufficiently promising to warrant an investigation of the value of this measurement throughout the entire period from germination to inflorescence.

EXPERIMENTAL

A sample of Government-certified Mt. Barker subterranean clover seed * was inoculated with Rhizobia ** and germinated in sand in spring. Two hundred seedlings taken at random as soon as the cotyledons emerged, were planted individually in plant pots containing unwashed sand, and grown in the glasshouse under the usual conditions of sand culture. They received weekly applications of a complete nutrient solution made up in tap water

* Supplied by Arthur Yates & Co. (Pty.) Ltd., Sydney.

** Strain RS.1.306/1 supplied by Professor J. Vincent of Sydney.

and were watered as needed. They were divided into two groups, receiving nitrate nitrogen in the nutrient solution at 11 ppm and 110 ppm respectively. From each group, twenty plants were harvested at 5, 8, 12, and 18 weeks after germination.

Another group of hundred seedlings was planted in sand in the open air similarly to those under glass. The original nitrate supply was 11 ppm N but this concentration was insufficient as the symbiosis failed to function adequately. Consequently the nitrate supply was raised to 55 ppm which produced a so rapid growth response.

RESULTS

The low-nitrogen plants (N11) were dependent largely on symbiosis for their nitrogen supply, and grew much more slowly, although coming into flower more rapidly than their counterparts on the higher nitrogen supply (N110). This allowed the study of different developmental rates on the coefficient, with plants of the same age. In practice it was found that the fact that the seed-

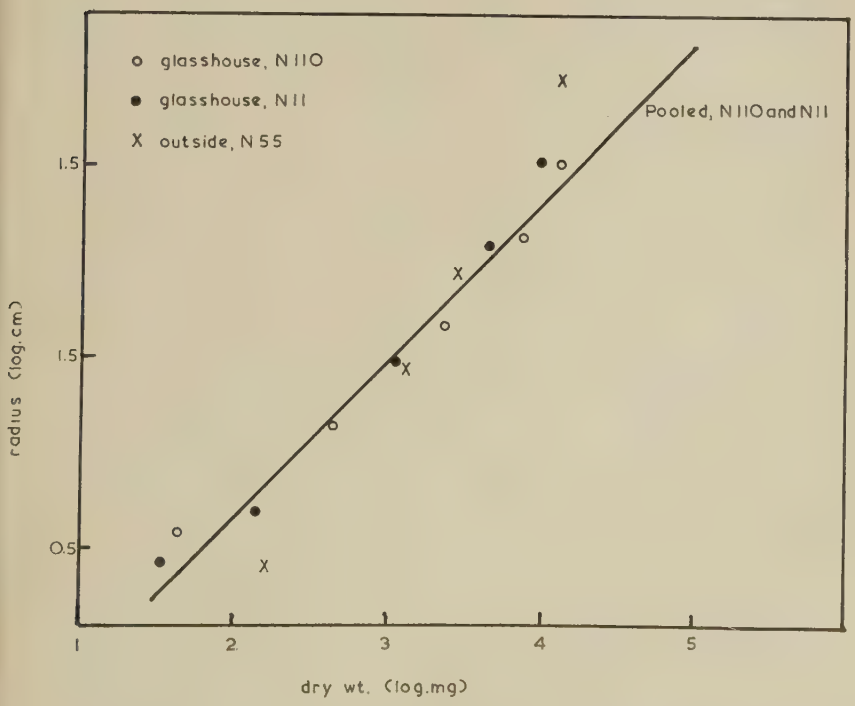

Fig. 2. Relationship of maximum radius and dry weight.

lings were unselected led to far greater variation in dry-matter yield than was obtained for the different nitrogen levels. For example, the dry matter of the twenty N110 plant tops at the end of the 14th. week ranged continuously from 3 to 12 g where as the mean dry weight of the two groups of plants were N11 = 5.3 g, N110 = 7.9 g. The results are summarised in Table 1.

If regression equations of the form $W = KR^n$ are fitted to the data from this experiment, it is found that where W is in mg and R in cm

for plants in the glasshouse, at N110 $W = 2.42\ R^{2.617}$ (1)
,, ,, ,, ,, ,, at N11 $W = 4.68\ R^{2.271}$ (2)
,, ,, ,, ,, open air at N55 $W = 14.41\ R^{1.485}$ (3)

All the n values are highly significant, but whilst those of the two groups grown in the glasshouse do not differ significantly, the differences between them and the coefficient for the outdoor plants is highly significant (at 1 per cent level). This is probably

TABLE 1

Correlation of dry weight of tops and maximum radius at different ages and nitrogen supply. Season: spring/summer 1957

Location	No. of plants	Nitrogen, ppm	Coefficient of correlation	Mean dry wt., g	Age, weeks	Development
Glasshouse	20	11	0.962	0.04	5	Early vegetative
			0.926	0.52	8	,, ,,
			0.700	1.2	12	Some early, some late vegetative
			0.877	5.3	14	Late vegetative, 1 plant in flower
			0.692	11.5	18	8 plants in flower
	20	110	0.984	0.05	5	Early vegetative
			0.947	0.55	8	,, ,,
			0.954	2.5	12	Internodes elongating
			0.902	7.9	14	Late vegetative
			0.703	15.0	18	4 plants in flower
	10	11	0.988		5	
	10	110	0.954		8	
			0.796		12	
Open air	19	55	0.973		9	Early vegetative
	19		0.870		11½	Some early, some late vegetative
	20		0.732		13	Rapid internode elongation, 4 plants in flower
	19		0.726		18	All plants in flower

because of differences in the growth form of the plants due to the differing climatic environments in which they have been grown, and for the index to be of use in the field it must be restricted to experiments which do not contain a climatic variable. The relationship between the regression line given by the equation $W = 3.70\ R^{2.427}$, calculated by pooling the data of Equations (1) and (2) above, and the harvest means for these three groups are given in Fig. 2.

DISCUSSION

The most practical requirement of a growth index in nutritional work is to reflect the *mean* dry weight of a treatment rather than the *individual* dry weights of its component replicates. That is, when a treatment effect is shown by the actual dry-weight data to be significant, the index should indicate when the effect first became significant. The maximum radius performed this rather better than might be expected. This was because the correlation coefficient was considerably higher when calculated from treatment means than from individual replicates. Using data from an experiment published elsewhere (Greenwood and Hallsworth [3], Experiment A), it was found that the value of r when calculated from treatment means was 0.96, but only 0.85 when calculated from replicates.

A more severe test of the fidelity of the maximum radius as an index of dry matter can be made by calculating parallel analyses of variance using first dry weights and then radii. This will distinguish very clearly between those variables whose yield effects are reflected by the maximum radius from those for which the index is invalid. Examples are given below in Tables 2 and 3 where data are taken from other experiments wherein different nutrients have been varied singly and in factorial combination.

Under conditions given in Table 2 it can be seen from the value of the variance ratio that differences between the 5 copper levels have been large and sufficiently well reflected by the index. At this stage, of course, such differences do not require an index to distinguish them as they are obvious. However, the small but significant differences between blocks has been accurately given by the index even at this advanced stage of growth.

TABLE 2

Comparison of variance ratios derived from dry wt. and index measurements. Plants grown symbiotically on 14 ppm N and complete nutrient. Coefficient of correlation calculated from means at harvest = 0.946. Age at harvest = 13 weeks. Season: spring/summer

Source of variance	Variance ratio	
	Dry wt.	Radius
5 copper levels	31***	21***
Blocks.	3.8*	2.6*

Table 3 presents data for $15\frac{1}{2}$ week-old plants which, on the evidence of Table 1 and from the fact that the coefficient was only 0.85 for individuals, are clearly too mature for the index to be expected to be accurate in such a severe test as the variance ratio. Table 3 is presented to show that this is indeed so, and that if it is intended to use the maximum radius for this degree of precision then the data at harvest must show the coefficient of correlation to be greater than 0.85 provided the plants are not flowering. Again on the evidence of Table 1 it can be considered that the younger the plant, the more certainty there is that the index will be sensitive, and it is in these early pre-harvest stages that an index is most required.

TABLE 3

Comparison of variance ratios derived from dry weight and index measurements. Plants grown a-symbiotically on 224 ppm N and Long Ashton nutrient solution. Coefficient of correlation for treatment means = 0.96 and for individuals = 0.85. Age at harvest = $15\frac{1}{2}$ weeks. Season: summer/autumn.

Source of variance	Variance ratio	
	Dry wt. (toos)	Radius
Ca64 — 8 ppm	49***	26***
Cu 0.064 — 0.0064 ppm	30***	12***
P40 — 8 ppm.	0.065	0.39
Ca × Cu.	5.214*	0.26
Ca × P	0.917	1.17
Cu × P	11.72**	8.83**
Ca × Cu × P	2.45	0.65

Another desirable attribute of a growth index is that estimates of dry weight may be made using a generalised regression equation. From Fig. 2, however, it can be seen that the maximum radius is not reliable enough to provide a *general* equation since differences between glasshouse and outdoor climate produced significant differences in the regression coefficient. In our experience with subterranean clover in England, the large seasonal variation in light intensity also alters the morphology of the plant to such an extent that the regression coefficient would be again significantly altered.

CONCLUSIONS

The maximum radius, taken as defined earlier, can serve as an excellent index of the dry weight for Mt. Barker strain of subterranean clover as long as the plants are in the early vegetative stage. It reflects dry-weight changes with greatest fidelity at the time when its use as a measurement of nutrient effects is most desirable.

It is most useful when the mean values for treatments, rather than the individual components of those means are employed. Analysis of the variance is also safe, at least for main effects, provided that the development of the plant is not advanced and that r for individuals might be expected to be greater than 0.9.

The index has been found, for experimental purposes, to have two virtues. In a large randomised block where it is impossible to compare small, significant yield effects visually other than by laboriously systematising the treatments, the comparison of the the radius means for treatments has been found to give a very faithful picture of both yield effects and of their relative dimensions. Without the tedium of intermediate harvests, it allows the construction of growth curves for comparison with environmental data.

Where it is essential for other reasons for more than one harvest to be taken, the comparison of radius means at the time of each harvest adds precision to the interpretation of nutrient effects.

Caution, of course, needs to be exercised in the interpretation of radius data, and when ultimately dry-weight values are obtained at the end of the experiment it is advisable to calculate the coefficient of correlation from a representative sample of individuals.

If this is relatively high, of the order of 0.8 or better then the radius can be safely used as an unbiassed index of dry weight, and the further one goes towards the beginning of the experiment, the more accurate the index will be.

If, however, r (individuals) is less than 0.8 then it appears advisable to calculate a parallel analysis of variance, and to determine from the variance ratios which of the variables is causing the bias. This in itself may contribute to the analysis of the results obtained.

SUMMARY

The maximum radius of the rosette, defined as the distance from the centre shoot to the base of the leaf furthest from it, is highly correlated with yield of dry matter ($r = 0.98$ to 0.93) over the earlier vegetative period, but becomes progressively less sensitive on approaching inflorescence.

The maximum radius was tested for fidelity as a rapid index of dry-matter yield using data from other factorial eperiments. It proved satisfactory in nutrient experiments for the comparison of treatment means prior to harvesting, provided that correlation at harvest was greater than $r = 0.85$.

The limitations of the index are discussed.

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REFERENCES

1 Aitken, Y., (1955) Flower initiation in legumes. Australian J. Agr. Research **6**, 212 (1955).

2 Anderson, A. J., Molybdenum and other fertilizers in pasture development on the Southern Tablelands of New South Wales. J. Australian Inst. Agr. Sci. **14**, 28 (1948).

3 Greenwood E. A. N. and Hallsworth E. G., Studies on the nutrition of the forage legumes. II. Some interactions of calcium, phosphorus, copper and molybdenum on the growth and chemical composition of *Trifolium subterraneum* L. Plant and Soil **12**, (in press) (1960).

4 Millikan, C. R., Nutritional disorders in subterranean clover. Dept. of Agr. Victoria Bull. No. **11** (1953).

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A STUDY OF SEVERAL FACTORS AFFECTING THE DISTRIBUTION OF PHOSPHORUS-32 FROM THE LEAVES OF *PISUM SATIVUM* *

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INTRODUCTION

Under normal conditions essentially all of the minerals which accumulate in the above-ground parts of plants are initially absorbed by the roots. Exceptions exist in agriculture when mineral solutions are applied to the foliage [17].

The accumulation of mineral solutes in leaves, subsequent to root absorption, may be followed by re-entry into the stem and movement upward and downward in the plant. This has been shown to be true for phosphate [3 5] and sulphur [6]. Similarly when a wide variety of solutes are supplied directly to leaves, export to other parts of the plant may occur [8]. In either case the phloem is thought to be the principal pathway for this transport [1 9].

Most studies on the translocation of mineral solutes from leaves have been concerned with relatively young plants (2 to 4 weeks old) and on plants lacking flowers or fruit. In this study the effect of flowers or of mature fruit of *Pisum sativum*, variety Alaska, on the movement of radioactive phosphate from leaves has been studied.

MATERIALS AND METHODS

Seeds of Pisum of uniform size were allowed to germinate in petri dishes, selected for uniformity after 48 hours, and planted in a soil:sand:peatmoss

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(3:2:1) mixture. Plants were grown in a controlled-environment room under a 12-hour photoperiod at 24°C (75°F), followed by a 12-hour dark period at 15.5°C (60°F). Under these conditions, two pods were usually produced on each plant and plants were used for the experiments at about 50 days after germination unless otherwise noted. At this time peas in the pods were mature.

The radioactive phosphorus was applied to the plants as $H_3P^{32}O_4$ in a drop application to a leaflet at selected nodes, following essentially the procedures used by Swanson and Whitney [16]. A single 10-lambda drop containing about 4 to 6 μc of the radioactive phosphate solution was applied to one leaflet of each test plant. Plants remained in the controlled-environment room throughout the period of absorption and translocation of the radioactive phosphate.

The uptake period was 3 to 4 hours unless otherwise indicated. At harvest the plants were cut into appropriate parts, dried in an oven, and digested with concentrated nitric acid in 50-ml beakers. After digestion was completed, the excess nitric acid was evaporated off and the beakers containing the radioactive residue were counted in a thin-window Geiger Counter. Counts were corrected for background and for decay where necessary. It should be emphasized that all counts are relative and can be compared directly only with counts made within the same experiment. The data were not corrected so that absolute values could be compared among different experiments. All experiments were repeated at least once.

RESULTS AND DISCUSSION

The distribution of phosphorus-32 as affected by steam-girdling the petiole

There is evidence in the literature that phosphorus-32 supplied to a leaf by the leaf-flap method [2] or by the leafdrop method [16] is translocated from the leaf by way of the phloem. In order to confirm this observation for Pisum an experiment was performed in which the petiole of the supply leaf (to which phosphorus-32 was applied on one leaflet) was steam girdled. This was accomplished by focusing a small jet of steam on a 1-cm segment of the petiole midway between the stipule and the basal leaflets, just prior to application of the phosphorus-32. At harvest each plant was divided into the following parts: the stem and leaves above the supply leaf (designated as St_A); the corresponding portion below this leaf (St_B); the pod in the axil of the supply leaf (P_1); and the second pod, when present, (P_2). (See Fig. 1).

The results of this experiment are given in Table 1. It is evident that scalding a short zone of the petiole of the supply leaf greatly

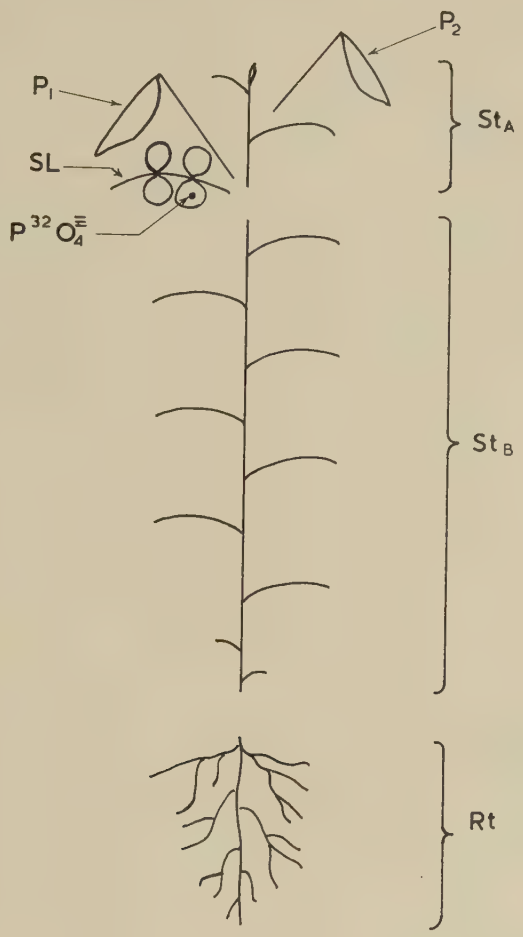

Fig. 1. General plan for separating the plants into parts for assaying. Designations for the several parts are as follows: **Rt**, roots; **St_B**, the stem and leaves below the first bloom node (in most experiments the tracer phosphorus was applied to the leaf at this node); **St_A**, the stem and leaves above this bloom node (this included the "supply" leaf except where noted); **P_1**, the first pod (including the peas except where these were counted separately as noted); **P_2**, the second pod; **SL**, the supply leaf, consisting of the petiole and blade except for the leaflet to which the P^{32} was applied (included with St_A where noted).

reduced the amount of labelled phosphate exported from this leaf (an average of 119 cpm) in $St_A + St_B + P_1$ of the petiole-scalded plants as compared with an average of over 30,000 cpm in the

corresponding parts of the control plants. The reduction, therefore, amounted to over 99.5 per cent. These data affirm the prevailing view that phloem blocking prevents, or at least greatly inhibits, the export of solutes from leaves. Petiole-girdled check plants

TABLE 1

The distribution of phosphorus-32 when the petiole of the "applied leaf" has been steam-girdled. Averages of 8 plants per treatment. (For abbrevations of plant parts see Fig. 1)

Treatment of petiole	Cpm/plant part			
	$St_A + St_B$	P_1	Leaf *	Total
None	6,160	23,078	1,198	30,436
Girdled	81	38	1,718	1,837

* Leaf portion consisted of the petiole and 3 leaflets external to the girdled zone. The "applied" leaflet was discarded.

were observed for several days and the leaflets external to the girdle did not wilt. Thus it appears that the xylem was not blocked by girdling.

The distribution of phosphorus-32 as affected by site of application

Phosphorus-32 was applied as a drop of solution to leaflets at different nodes, in order to learn which leaves were a source of supply to the developing fruits as well as for the roots and other portions of the plant. Distribution periods of from 4 to 7 hours were permitted before harvest. The harvest plan followed that described in Figure 1.

TABLE 2

The distribution pattern of phosphorus-32 in plants bearing a pod at the tenth node only. Radioactive phosphate applied to a leaf either at seventh or tenth nodes. Averages of 12 and 16 plants respectively. (For abbrevations of plant parts see Fig. 1)

Applied node	Counts per minute/plant part					% of total activity/plant part			
	Rt	St_B	St_A	P_1	Total	Rt	St_B	St_A	P_1
10	3	447	809	2543	3802	0.0	11.8	21.3	66.9
7	2	2294	1633	30	4016	1.5	57.1	40.7	0.8

The distribution patterns for phosphorus-32 when applied at the 10th or bloom node and at the 7th node are given in Table 2. Only a small percentage of the total radioactivity in the plant was

found in the pod at the 10th node (P_1) when the phosphorus-32 was applied to a leaflet at the 7th node compared with an average accumulation of about 67 per cent when movement of the labelled phosphate came from a leaflet at the first bloom node. This striking specificity was noted in all subsequent experiments, the percentage accumulation by the first pod ranging from 50 to 90 per cent in different experiments, whereas less than 1 per cent of the totaal activity in the plant was found in this pod when the phosphorus-32 was applied at the 7th node. In another series of plants having three pods the same specificity of movement from the first bloom node leaflet to the pod at that node was revealed, as shown in Table 3. Essentially no transport occured to either the second or

TABLE 3

The distribution pattern of phosphorus-32 in plants bearing pods at the eleventh, twelfth, and thirteenth nodes. Radioactive phosphate applied to a leaf either at seventh or eleventh nodes. Averages of 3 plants for each group

Applied node	Counts per minute/plant part						
	Rt	St_B	St_A	P_1	P_2	P_3	Total
7	1491	3068	1056	4	3	1	5623
11	573	538	3781	19048	11	7	23959

third pods (P_2 and P_3) from the leaf at the 11th node. Within counting errors the activity could have been recorded as zero for any of the pods on the plants which received phosphorus-32 at the 7th node. In these plants, however, more activity was recovered in the roots (Rt) and in the stem and leaves (St_B) portion.

Considerably less total activity was recovered in plants receiving the phosphorus-32 at a 7th node leaflet and this may have been due to the older physiological age of the supply leaf. Lower leaves of Pisum characteristically senesce and wither as the plant matures, although in these experiments the 7th leaf was still green and turgid. A summary diagram showing the distribution pattern of phosphorus-32 in relation to the several parameters mentioned above is presented in Figure 2.

The effect of the developmental stage of the first flower on the distribution of phosphorus-32

In the experiments discussed thus far the fruit was at or near maturity. In order to learn what the distribution pattern is in

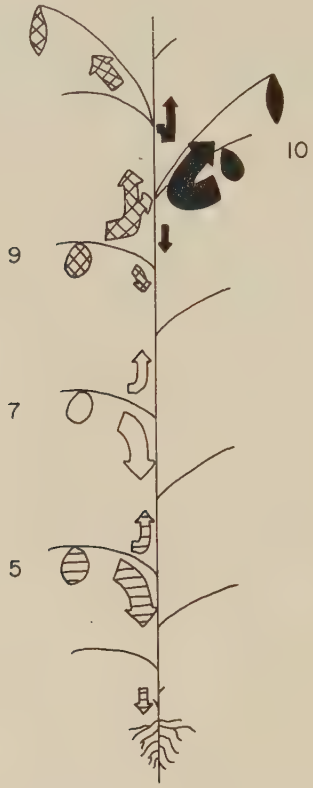

Fig. 2. A summary diagram of the distribution of phosphorus-32 when applied at different leaf nodes of the pea plant. Individual experiments are indicated by different markings on the arrows. Size of the arrows is roughly indicative of the magnitude of the phosphorus-32 movement in the direction shown.

plants bearing flowers in early stages, two experiments were performed in which the age of the flower at the first bloom node was 1 day pre-anthesis and 4 days postanthesis, respectively. Fertilization in this variety of Pisum probably occurs at anthesis or the day following [13]. Phosphorus-32 was applied to the leaflet at the first bloom node and following a three-hour absorption period the plants were divided into the St_A and St_B portions as before, and in addition the flower at the first bloom node was divided into calyx, corolla, stamens, and ovules. The "supply leaflet" was discarded and the remaining parts of this leaf (Lf) were harvested and counted separately. The results are given in Table 4. For

TABLE 4

The distribution of phosphorus-32 from the leaf at the first bloom node when the flower at that node is in the stage one day before anthesis or in the stage four days past anthesis. Averages based on 5 and 6 plants respectively

Stage	Cpm/plant part							
	St_B	St_A	Leaf	Petals	Sepals	Stamens	Ovules	Carpel
1 day before anthesis	20	5	3954	1	1	0	0	1
4 days after anthesis	2821	3837	1306	— *	1306	— *	3217	20,446

* Plant parts had abscised.

plants at either flower stage a considerable amount of activity was recovered in the remainder of the "supply leaf", thus suggesting roughly comparable absorption rates. The activity recorded for the flower parts and for the stem-above portion (St_A) in the plants at 1 day pre-anthesis was not above expected counting errors, and an average of only about 20 cpm for the stem-below (St_B) was recorded. These values should be compared with the rather large amounts of activity found in all parts of the flower and in the vegetative parts of the plant having a first bloom-node flower 4 days post anthesis. Although the embryo is still in a stage having but a few cells, the effect on distribution both to the flower itself and to other parts of the plant above and below the node of application is very marked. A similar distribution pattern was obtained in a subsequent experiment where the flowers at the first bloom node were 4 to 6 days old.

The effect of pod temperature on the distribution of phosphorus-32

Because there is a marked specificity for accumulation by the first pod when phosphorus-32 is applied to the leaf at this same node and because the age of this organ appears to be very important in its influence on distribution patterns, it was thought that the effect of pod temperature on phosphorus-32 transport might be of interest. Numerous workers have studied the effect of temperature on translocation. There appears to be an optimum temperature range (20° to 30°C) for the movement of carbohydrates [11 15] and for several inorganic ions [16], the rate or amount of movement decreasing at temperatures above or below this general "optimal" range.

In the studies here, only the effect of temperature of the pod itself was studied. The desired pod temperature was obtained by inserting the pods into metal or plastic jackets like those used by Swanson and Whitney[16]. The air temperatures within the jackets and the pod temperatures were checked by means of thermocouples. In all experiments the jackets were placed around the first bloom-node pod 30 to 60 minutes before the phosphorus-32 was applied to a leaflet at this node. This period was found to be satisfactory for equilibration of the pods with the air temperature produced within the jackets. As a check, jackets (of "high" temperature type) were placed on some pods, but not closed at the ends so that pods remained at the ambient temperature. It should be emphasized that all plants (regardless of pod treatment) were subjected to an ambient temperature of 26°C and only the pod at the first bloom node received the higher or lower temperature. The supply leaf in these experiments was the leaf at the first bloom node. The harvest plan followed that shown in Figure 1, with one

TABLE 5

The effect of the temperature of the first bloom-node pod on the distribution of phosphorus-32 from a leaflet at the same node. Averages based on three plants per treatment

Plant temp. C.	Pod temp. C.	Cpm per plant part				% of total activity		
		St_B	St_A	P_1	Total	St_B	St_A	P_1
26	7	763	3453	224	4440	17.2	77.8	5.0
26	26	1446	14647	11753	27846	5.2	52.6	42.2
26	36	2077	10120	23033	35230	5.9	28.7	65.4

TABLE 6

The effect of the temperature of the first bloom-node pod on the distribution of phosphorus-32. Data from plants having two pods. Averages based on 5 plants per treatment

Plant temp. °C	Pod temp. °C	Cpm per plant part						% of total activity			
		St_B	St_A	P_1		P_2	Total	St_B	St_A	P_1	P_2
				Pod	Peas						
26	7	1576	2171	2496	1532	396	8081	19.5	26.9	49.9	4.8
26	26	690	1523	1374	5878	2	9467	7.3	16.1	75.6	0.0
26	39	712	1756	2163	6022	2	10655	6.7	16.5	76.8	0.0

modification in the last two experiments where the peas were counted separately. A total of five experiments were performed

and the data from two will be given here (Tables 5 and 6). When there was only one pod on the plant and when its temperature had been lowered, less phosphorus-32 was translocated from the applied leaflet to the entire plant (Table 5). For example, the total activity in each of the 3 plants, with first bloom-node pods at 7°C, was approximately 4000 cpm compared to total counts up to 27,000 cpm in a plant whose first bloom node remained at room temperature (26°C). These results are converse to expectations inasmuch as with less phosphorus-32 accumulating in the pods, more phosphorus-32 should have been available for transport elsewhere in the plant. It is also of interest to note that this inhibition occurred even though the pod was outside of the direct line of transport to other areas of the plant. Böhning *et al.* [7] found a similar "remote control" effect of cooling the hypocotyl of bean plants on movement of carbohydrate to the growing stem tip. In the present experiments, this "remote control" effect was nullified, however, if a second pod was present on the plant, as may be seen from the data in Table 6.

As might be expected, lowering the metabolic activity of the pod itself resulted in the accumulation of less phosphorus-32 by the fruit. This reduction is largely due to the reduced movement of phosphorus-32 to the seeds themselves, rather than to the carpel tissue (Table 6).

The lower total activity for the plant when the first bloom-node pod was cooled can be accounted for largely in the reduced accumulation of phosphorus-32 by the pods themselves and also, to a lesser extent, by lower amounts of labelled materials in the St_A and St_B portions (Table 5). As noted above, when a second pod was present, greater amounts of phosphorus-32 were translocated to the second pod (P_2) and to the St_A portion of the plant compared to the check plants (plants at 26°C, including pod P_1; Table 6). The greater amount of phosphorus-32 found in the St_A might be expected since this portion includes the phloem pathway to the second pod.

Maximum accumulation of labelled phosphorus occurred in the pods at the highest temperatures, although it is likely that these temperatures exceeded the optimum for translocation. This situation resembles that reported by Swanson and Whitney [16] who observed that maximum accumulation of phosphorus-32

occurred in localized temperature zones on bean plants at temperatures considerably in excess of those favoring maximal rates of translocation. It should be recalled that these temperature treatments were of a short duration (3 to 4 hours) and over more extended periods, "high" temperatures might have a deleterious effect.

These results indicate a marked specificity in the translocation of phosphorus-32 from a given leaf to both vegetative and reproductive parts of the plant. When pods are present the movement of labelled phosphorus to these organs accounts for the bulk of the activity recovered in the plant when it is applied to a bloom-node leaf. This distribution pattern exists from the time the flower is pollinated and apparently continues through early embryonic stages of the peas until they are mature. This distribution pattern was seen in those experiments where only a relatively small amount of phosphorus-32 moved into any part of the plant from the first bloom-node leaf before anthesis had occurred in that flower. Evidently the pattern of distribution of phosphorus to a given pod from the leaf at that bloom node is established shortly following anthesis. It seems possible that auxin produced by the embryo may play an important role in "controlling" the movement of solutes to the pod and to other parts of the plant as well as to the embryos themselves. Biddulph and Brown [4] have reported a direct relationship between synapsis and syngamy and dry-weight accumulation in cotton fruit. They also found that there was an increase in phosphate accumulation after both synapsis and syngamy. Nitsch [14] has demonstrated, for strawberry, the role of the embryo in producing auxin which is responsible for the morphological development of accessory tissues. The temperature data presented above also suggest that metabolic activity is involved. When the pod at the first bloom node was cooled (to about 7°C), the movement to this pod and to other parts of the plant was greatly reduced, even though the plant excluding the pod was at a metabolically favorable temperature (about 25°C). In thus appears that the pods are a controlling factor, metabolically, in the distribution of solutes through the phloem.

To what extent these conclusions may be applied to the transport of other solutes from the leaves, such as sugars, is not known, although evidence presented in the literature [1 9 12] suggests that

radioactive phosphate applied to a leaf is exported only under conditions when export of photosynthate may also be expected. It is possible, therefore, that foliage-applied P^{32} may be used to indicate roughly the distribution patterns of photosynthate, at least in short-term studies (translocation periods of a few hours, as in the present experiments). Differences in rates of radial transfer from the phloem to the xylem and in the rates of re-cycling within the plant may be expected to complicate the comparative distribution patterns in time.

SUMMARY

1. Distribution patterns for the movement of solutes in the phloem from leaves of pea plants were found to be relatively specific. Phosphorus-32 applied to the leaf at the first bloom node moved predominantly (to the extent of 50 to 90 per cent) to the pod at that node. Distribution of the translocate from this leaf to pods at higher nodes was negligible.

2. Translocation in the phloem of phosphorus-32 from lower leaf nodes (*i.e.* 5th or 7th) was predominately downward with little or no accumulation in the pods.

3. The stage of development of the flower markedly affected the distribution pattern of phosphorus-32 supplied to the leaf at the same node. Essentially no activity moved into either the flower or the vegetative portions of the plant before anthesis and fertilization had occurred in that flower, as compared to the same plant parts in plants 4 to 6 days past anthesis. The young developing embryos of the pod appear to "control" the movement of phosphorus-32 from the adjacent leaf.

4. When the metabolic activity of the pod on plants bearing one pod only was lowered by cooling (to 7°C), the movement of phosphorus-32 to this pod as well as to all other parts of the plant was markedly diminished. This inhibitory effect of the low pod temperature on translocation to the uncooled parts of the plant was negated by the presence of a second pod (uncooled) on the plant. Lowering the temperature of the first pod resulted in a substantial increase in the proportion of the P^{32}-labelled translocate moving to the second pod.

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LITERATURE CITED

1 Biddulph, O., Absorption and movement of radiophosphorus in bean seedlings. Plant Physiol. **15**, 131–136 (1940).

2 Biddulph, O., Diurnal migration of injected radiophosphorus from bean leaves. Am. J. Botany **28**, 348–352 (1941).

3 Biddulph, O., Biddulph, Susann, Cory, R., and Koontz, H., Circulation patterns for phosphorus, sulfur, calcium in the bean plant. Plant Physiol. **33**, 293–300 (1958).

4 Biddulph, O. and Brown, D. H., Growth and phosphorus accumulation in cotton flowers as affected by meiosis and fertilization. Am. J. Botany **32**, 182–188 (1945).

5 Biddulph, O., Cory, R., and Biddulph, S., The absorption and translocation of sulphur in red kidney bean. Plant Physiol. **31**, 28–33 (1956).

6 Biddulph, O. and Markle, Jane, Translocation of phosphorus-32 in the phloem of the cotton plant. Am. J. Botany **31**, 65–70 (1944).

7 Böhning, R. H., Swanson, C. A., and Linck, A. J., The effect of hypocotyl temperature on translocation of carbohydrates from bean leaves. Plant Physiol. **27**, 417–421 (1952).

8 Bukovac, M. J. and Wittwer, S. H., Absorption and mobility of foliar applied nutrients. Plant Physiol. **32**, 428–435 (1957).

9 Colwell, Robert N., The use of radioactive phosphorus in translocation studies. Am. J. Botany **29**, 798–807 (1942).

10 Day, B. E., The absorption and translocation of 2,4-dichlorophenoxyacetic acid by bean plants. Plant Physiol. **27**, 143–152 (1952).

11 Hewitt, S. P. and Curtis, O. F., The effect of temperature on loss of dry matter and carbohydrate from leaves by respiration and translocation. Am. J. Botany **35**, 746–755 (1948).

12 Kendall, W. A., Effect of certain metabolic inhibitors on translocation of P_{32} in bean plants. Plant Physiol. **30**, 347–350 (1955).

13 Linck, A. J., Studies on the distribution of phosphorus-32 in *Pisum sativum* in relation to fruit development. Diss. Abs. **XV**, 951–952 (1955).

14 Nitsch, J. P., Growth and morphogenesis of the strawberry as related to auxin. Am. J. Botany **37**, 211–215 (1950).

15 Swanson, C. A. and Böhning, R. H., The effect of petiole temperature on the translocation of carbohydrates from bean leaves. Plant Physiol. **26**, 557–564 (1951).

16 Swanson, C. A. and Whitney, J. B., Studies on the translocation of foliar-applied phosphorus-32 and other radioisotopes in bean plants. Am. J. Botany **40**, 816–823 (1953).

17 Wittwer, S. H., Nutrient uptake with special reference to foliar absorption. Atomic Energy and Agriculture V, pp. 139–164. Published by Am. Assoc. for Adv. Sci. **49**, (1957).

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STUDIES ON THE DECOMPOSITION OF AMINO ACIDS IN SOILS

II. THE ANAEROBIC METABOLISM

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INTRODUCTION

The finer pore spaces of a soil in humid, temperate, regions of the Earth can rarely be free of water under natural conditions. Within the interior of the soil crumbs indeed access of oxygen must be greatly impeded by the presence of this water, and conditions must be anaerobic for a considerable part of the year. The extent of anaerobic metabolism, potential or actual, even in soils normally considered to be well aerated, is perhaps evidenced by the universal occurrence of strictly anaerobic micro-organisms in such soils.

This paper describes the results of experiments on the anaerobic breakdown of the common amino acids in soil, a process which seems hitherto not to have been comprehensively studied despite its importance to our understanding of the anaerobic biochemical economy of soils.

MATERIALS AND METHODS

Soil

The soil investigated was the garden soil used in previous work [10 11] on the aerobic breakdown of amino acids. It was a sandy loam, 0–25 cm,

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C = 3.7% [25], N = 0.4% (by Kjeldahl), and pH = 6.5 at a soil: water ratio of 1 : 2 (*w/v*).

Preparation of soil extract

A water suspension of garden soil (900 ml : 400 g) was shaken for 60 min, centrifuged at 400 × *g* and filtered through No. 1 Whatman filter paper. The resultant filtrate was used as "soil extract". The procedure was adopted to ensure an adequate distribution of microbial inoculum throughout the mixture.

Anaerobic incubation

This was carried out under N_2, H_2, or CO_2. Soil extract (25 ml) and 6 g of soil were transferred to a 40 ml. boiling tube and thoroughly mixed with a known weight of the substrate. The boiling tube was then plugged with non-absorbent cotton-wool and transferred to a Fildes jar. After the jar had been evacuated and outgassed with the appropriate gas (H_2, N_2, or CO_2) it was incubated in the dark at 23°C for ten days. A glucose/methylene blue/NaOH mixture [19] was used in the jar to check that anaerobic conditions were being maintained. After 10 days' incubation, a 1- or 2-ml sample ("the aqueous extract") was taken from each boiling tube and the remainder of the incubate extracted with K_2SO_4/H_2SO_4. Controls were always run with soil and soil extract but without substrate and all data presented have been corrected for these 'blank' values.

Extraction of cations

The method of extraction was based on that used by Bremner and Shaw [2]. Sufficient 2 *N* H_2SO_4 (1 ml) and K_2SO_4 (2 g) were added to each boiling tube to bring the resulting mixture to pH 1 and to make it Normal with respect to potassium. After 30 min shaking, the mixture was filtered and the supernatant used for the α-amino-N, NH_3-N, and volatile fatty acid determinations.

Determination of the degree to which amino acids were adsorbed on the soil surface

In order to test the degree to which amino acids were adsorbed on the soil, *e.g.* by the base-exchange complex, each amino acid (20 mg) was shaken at 120 cycles/min for 30 min with garden soil (6 g) and water (25 ml). The resulting suspension was filtered and the filtrate analysed for α-amino-N.

α-Amino-N

The method used for these determinat ons was essentially that of Woiwood [24]. Some analyses were made on samples from the aqueous extract and account was then taken of the amino-acid adsorption on the soil surface.

Most analyses were, however, made on samples of the K_2SO_4/H_2SO_4 extract which removed from the soil more than 93% of all added amino acids.

Ammonia-N

This was determined on samples of the K_2SO_4/H_2SO_4 extract which completely removed NH_3-N from the soil surface. Most estimations were made on 1- or 2-ml samples, containing < 100 μg NH_3-N, by the method of Conway [6]. A few estimations were made on 1- to 5-ml samples containing 30 to 500 μg NH_3-N by distillation from 40% KOH in a Markham apparatus [17]. The distillate was collected in 5 ml 1% boric acid containing 40 ml Conway's [7] mixed indicator per litre and titrated with 0.007 *N* HCl.

Indole

Indole was determined on 0.2 to 0.6 ml of the aqueous extract by the method of Happold and Hoyle [12]. In assessing the total indole present in the soil incubate, account was taken of adsorption of indole on the soil surface.

Nitrate- and Nitrite-N

Both determinations were made on samples of the aqueous extract by the colorimetric methods described by Lees and Quastel [14].

Volatile fatty acid

A 5-ml sample of the K_2SO_4/H_2SO_4 extract, was distilled with 5 ml of 3 *N* H_2SO_4 in a Markham distillation apparatus [17]. Distillate (60 ml) was collected in a receiver and titrated with 0.01 *N* $Ba(OH)_2$ with 0.3 ml of 0.01% phenol red in 25% ethanol, adjusted to pH 8, as indicator. During titration, the distillate was stirred by a stream of CO_2-free air. Control experiments, to test the immediate recovery (by extraction with the K_2SO_4/H_2SO_4 mixture) of acetic and butyric acids added to soils showed minimal recoveries of 92 per cent; therefore not more than 8 per cent of these acids was held on the soil surface under these conditions. Nitrite, but not nitrate, was found to interfere with the volatile fatty acid determinations. Where nitrite was present in quantity it was therefore removed, by treatment of a 5-ml sample with 2 ml of 2 *N* $KMnO_4$ in *N* H_2SO_4 for 2 min in the cold and then with excess solid $FeSO_4$ to decolorize the remaining $KMnO_4$, prior to the determination of volatile fatty acid.

Chromatography

Samples of the aqueous extracts were analysed by one-dimensional paper chromatography; the solvent used was either water/butanol/acetic acid (5 : 4 : 1 by vol.) or phenol/water (4 : 1 *w/v*). Occasionally two chromatograms, one in each solvent, were run on a sample. The spots were developed by spraying with a 0.1% ninhydrin solution in butanol.

RESULTS

Table 1, which gives the mean results of two experiments, illustrates the pattern of amino-acid breakdown after ten days of anaerobic incubation in a garden soil under nitrogen. The results of replicate experiments on the same soil sampled at different times of year confirmed the general pattern of those in Table 1, although the absolute rates of breakdown of the different amino acids varied slightly. The "resistant" amino acids, *i.e.*, hydroxyproline, leucine, methionine, phenylalanine, proline, threonine, tryptophan, tyrosine, and valine always decomposed more slowly than did alanine, arginine, aspartic acid, cystine, glutamic acid, glycine,

TABLE 1

Anaerobic decomposition of amino acids

The figures for α-amino–N, NH_3–N and volatile fatty acid were obtained after 10 days incubation of approx. 20 mg of each amino acid with 6 g of garden soil and 25 ml of soil extract, under N_2, at 23°C. Volatile fatty acid is expressed per 100 μmole of added amino acid. The figures for α-amino–N and NH_3–N indicate the percentage of the initial amino acid remaining at the end of the experiment and the percentage nitrogen of the amino acid converted to NH_3–N

Amino acid	μ-equiv. volatile fatty acid	NH_3–N %	α-Amino–N %
DL-alanine	87	81	0
L-arginine. HCl	106	77	0
L-aspartic acid	62	72	0
L-cystine	93	81	0
L-glutamic acid	99	78	0
Glycine	47	79	0
L-histidine. HCl	82	73	0
L-hydroxyproline	12	17	77
DL-leucine	7	14	84
L-leucine	8	18	76
L-lysine. HCl	142	76	0
DL-methionine	5	20	76
L-methionine	10	23	71
DL-phenylalanine	10	15	78
L-phenylalanine	7	16	75
L-proline	7	14	82
DL-serine	64	84	0
DL-threonine	22	26	71
L-threonine	26	26	74
L-tryptophan	33	26	50
L-tyrosine	15	5	—
DL-valine	6	15	83
L-valine	5	18	76

histidine, lysine, and serine. Decomposition was accompanied by the formation of volatile fatty acid.

Except for the nitrogen of tryptophan, 80 per cent of the nitrogen of the amino acid decomposed can be accounted for as ammonia. When tryptophan decomposed the nitrogen appeared in the form of indole as well as of ammonia and these two products were formed in approx. equi-molar amounts. The average standard deviations in the figures for volatile fatty acid and ammonia produced, were for those amino acids that completely decomposed, 9.7 μ-equiv./100 μmole amino acid (27) and 2.9% N (27) of the amino acid respectively. Most amino acids decomposed with the formation of approx. 1 equiv. volatile fatty acid per mol. of amino acid metabolised, although glycine yielded approx. 0.5 equiv. per mol. and lysine and glucose (Table 4) both yielded approx. 1.5 equiv. per mol. Chromatography showed no ninhydrin-positive spots other than those corresponding to unchanged amino acids. No volatile amines were detected. Estimations of ammonia by the Conway technique [6] agreed with those obtained by the use of the Markham apparatus [17] which estimates both amines and ammonia. Amino-acid breakdown under H_2 or CO_2 followed the same pattern as breakdown under N_2, except that under H_2 more volatile fatty acid was produced than under N_2 or CO_2.

The breakdown of the "resistant" amino acids was accelerated when they were in combined form as in casein or in a suitable complex mixture with other compounds as in casein hydrolysate. Experiments in which casein (30 mg) was incubated under N_2 with soil (6 g) at 23° C for twelve days showed that at no time during the incubation could α-amino-N be detected in concentrations > 0.6 per cent of the added casein-N, although 50 per cent of the casein-N was finally converted to NH_3-N. Similarly when acid casein hydrolysate (30 mg) was incubated under N_2, with soil (6 g) at 23° C no α-amino-N could be detected after 5 days.

The slow rates of breakdown of the "resistant" amino acids cannot be attributed to the effects of optical isomerism for both DL and L forms of these amino acids decomposed to the same extent (Table 1). Nor can the resistance or non-resistance to breakdown be attributed to the effects of adsorption on the soil surface (Table 2). Of the amino acids appreciably adsorbed (arginine, histidine, lysine, and tryptophan) tryptophan was "resistant" and

TABLE 2

Adsorption of amino acids by garden soil

The percentage of the amino acid adsorbed on the soil surface when 25 mg of the indicated amino acid dissolved in 25 ml of water were shaken with 6 g of garden soil

Amino acid	% adsorbed	Amino acid	% adsorbed
DL-alanine	8	L-arginine. HCl	71
L-aspartic acid	9	L-glutamic acid	6
Glycine	12	L-histidine. HCl	53
L-leucine	10	L-lysine. HCl	58
DL-methionine	8	L-phenylalanine	11
L-proline	8	DL-serine	8
DL-threonine	10	L-tryptophan	36
DL-valine	10	0.005N(NH_4)$_2SO_4$(25 ml)	50

the rest were not; a similar situation exists among the non-adsorbed amino acids.

A possible explanation for the resistance to breakdown of some amino acids when added singly to soil was that under these con-

TABEL 3

Anaerobic decomposition of mixtures of amino acids

The percentage of the added amino acid(s) remaining unchanged after 10 days incubation with 6 g of garden soil and 25 ml of soil extract under N_2 at 23°C. Approximately 100 μmole of L-leucine, L-methionine, L-phenylalanine, and L-valine and approximately 200 μmole of L-proline and glycine were used. Chromatograms indicated the presence of all added amino acids except glycine; no other spot appeared. Glycine disappeared with the formation of approx. 0.85 mol. NH_3–N/mol. and 0.5-equiv. volatile fatty acid/mol.

Amino acid(s)	% α-amino-N
Glycine	0
L-leucine	85
L-methionine	92
L-phenylalanine	90
L-proline	77
L-valine	90
L-leucine + L-proline	68, 68
L-phenylalanine + L-proline	80, 80
L-valine + L-proline	75, 75
L-leucine + Glycine	89, 0
L-methionine + Glycine	94, 0
L-phenylalanine + Glycine	83, 0
L-valine + Glycine	71, 0

ditions the Stickland reaction [21] could not operate while with mixtures of amino acids it could. This hypothesis was tested by following the breakdown of appropriate mixed pairs of L-amino acids including tryptophan and leucine, tryptophan and phenylalanine, tryptophan and methionine, tryptophan and alanine, and threonine and proline. In all cases the results were similar to those in Table 3 which is derived from a typical experiment. The data demonstrate that the presence or absence of a suitable "Stickland pair" [21] amino acid makes no difference to the breakdown of the "resistant" amino acids under the conditions of experiment. An amino acid that breaks down slowly when added singly to the soil continues to breakdown slowly in the presence of a suitable "Stickland pair" [21] amino acid irrespective of whether this latter amino acid itself breaks down slowly or quickly.

Since many organisms can derive their energy by the metabolism of glucose, and since glucose is known to accelerate the aerobic breakdown of methionine [10 11] and threonine [10] in soil its effect on anaerobic amino acid breakdown was examined. Table 4 shows that breakdown of the "resistant" amino acids was only slightly

TABLE 4

Effect of glucose on anaerobic decomposition of amino acids

The percentage of amino acid remaining unchanged after 10 days incubation of the amino acid (approx. 20 mg), in presence and absence of glucose (approx. 20 mg), with 6 g of garden soil and 25 ml of soil extract under N_2 at 23°C. All glucose added decomposed with the formation of approx. 1.5 equiv. volatile fatty acid/mol.

Substrate	% α-amino–N
DL-leucine	80
DL-methionine	87
DL-phenylalanine	80
DL-threonine	71
DL-tryptophan	32
DL-valine	92
DL-leucine + glucose	56
DL-methionine + glucose	72
DL-phenylalanine + glucose	75
DL-threonine + glucose	63
DL-tryptophan + glucose	28
DL-valine + glucose	72

accelerated by the concomitant decomposition of an equal weight of glucose.

The results of a typical experiment showing the effect of nitrate on the metabolism of amino acids is shown in Table 5. The breakdown of the "resistant" amino acids, in the presence of nitrate was somewhat accelerated but still rather slow. With the exception of DL-methionine, all the "resistant" amino acids tested acted as hydrogen donors for nitrate reduction. Nitrite-N so

TABLE 5

Effect of nitrate on the anaerobic decomposition of amino acids

Analyses expressed per 25 ml of extract, after fermentation of the indicated amounts of amino acid for 10 days with 6 g of garden soil, 25 ml of water or 25 ml of 0.02 *M* KNO_3 under N_2 at 23°C

Amino acid	α-amino–N added	α-amino–N	NH_3–N	NO_3^- + NO_2–N	NO_2–N	Volatile fatty acid
	mg	mg	mg	mg	mg	μ-equiv.
DL-alanine	3.54	0.00	3.00	0.0	0.0	198
DL-leucine	2.08	1.70	0.22	0.0	0.0	6
DL-methionine	1.90	1.88	0.14	0.0	0.0	4
DL-phenylalanine	1.65	1.40	0.12	0.0	0.0	4
DL-threonine	2.30	2.03	0.27	0.0	0.0	10
DL-valine	2.56	2.23	0.10	0.0	0.0	4
DL-alanine and NO_3	3.70	0.00	2.47	0.1	0.1	0
DL-leucine and NO_3	2.18	1.04	0.73	3.4	3.3	3
DL-methionine and NO_3	2.05	1.86	0.23	6.8	0.1	0
DL-phenylalanine and NO_3	1.86	0.75	0.57	0.5	0.1	4
DL-threonine and NO_3	2.47	0.58	1.30	3.6	0.0	4
DL-valine and NO_3	2.52	0.70	1.30	0.0	0.1	10
No addition and NO_3	0.00	0.00	0.00	7.0	0.0	0

produced sometimes accounted for as much as 50 per cent of the total added nitrate-N, and these high concentrations were not specific for any amino acid. The reduction of nitrate always suppressed the formation of volatile fatty acid. Ammonia-N never exceeded the amino-acid nitrogen originally added to the incubate.

DISCUSSION

In experiments covering all the common amino acids, we have found that some of these acids, termed "resistant", decomposed much more slowly than the others when individually incubated under anaerobic conditions with garden soil (Table 1). When these

"resistant" amino acids were added as components of acid casein hydrolysate or of casein, however, they decomposed readily, despite the fact that amino-acid analyses [13] of casein show about 44 per cent of the component amino acids are "resistant". The slow rates of breakdown of the "resistant" amino acids could not be explained either by possible effects of adsorption on the soil surface (Table 2), or by interference (Table 3) with some normally operating Stickland reaction [21]. It seems therefore that the micro-organisms effecting anaerobic breakdown of some of the common amino acids in soil, may require a complex mixture of substrates for growth. It is interesting to note parenthetically that two of the "resistant" amino acids – methionine and threonine – also decomposed aerobically more slowly in the garden soil used in the present experiments than any of the other amino acids then tested [10 11]. The aerobic decomposition of methionine and threonine in soil was accelerated by glucose [10 11]; glucose under anaerobic conditions however failed to accelerate the decomposition of methionine or threonine or of any other "resistant" acid (Table 4). The reasons for the slow rates of breakdown of the "resistant" amino acids are not clear since the acids are neither structurally similar nor metabolically closely linked.

Since the biochemical conditions within the soil in our experiments have some resemblance to those obtaining in a rumen (mixed microbiological population, anaerobiosis, neutral pH), it is perhaps interesting to compare what happens in the rumen with what happens in soil. Lewis [15] has made an extensive study of the breakdown of amino acids in the rumen, his experiments involved incubating amino acids with suspensions of rumen organisms for a few hours. He found that amino acids when added individually as substrates broke down at different relative rates and that breakdown was accompanied by the formation of ammonia and volatile fatty acid. Operation of the Stickland reaction was very limited, although more rapid amino-acid breakdown resulted when the number of amino acids in the substrate was increased. He could, however, find no group or number of amino acids that specifically ensured rapid amino-acid breakdown. Despite the obvious similarity between Lewis's results [15] and our own the analogy between rumen and anaerobic soil should not be pushed too far. The microflora of the rumen is different from that of an anaerobic soil,

moreover in Lewis's studies [15] the amino-acid breakdown was effected by an approximately resting cell suspension whereas in the soil it was probably effected by proliferating cells, but the comparison remains interesting.

Adsorption of the amino acids by the soil surface (Table 2) does not seem to affect either their aerobic [23] or their anaerobic decomposition; arginine, histidine, and lysine are all strongly adsorbed by the garden soil yet decomposed readily aerobically [10 11], and anaerobically. The amino acids may here behave differently from certain other compounds, since there are many reports that the enzymic or microbial degredation of complex compounds, proteins and organic phosphorus compounds is slowed down by the presence of clays [1 8 9 16 18 20 22]. It has been suggested that this protection results in part, at least, from the adsorption of the substrate by the clay particles.

The results of the nitrate fermentation experiments seem to be consistent with the findings of other workers, namely that nitrate reduction under anaerobic conditions is dependent on the type of organic materials present [4], sometimes takes place with the temporary accumulation of large quantities of nitrite [3], and within ten days produces little free ammonia nitrogen [5].

Most of the amino acids not appreciably de-aminated anaerobically over a period of 240 hours were, by contrast, deaminated under aerobic conditions in the same soil within 24 to 48 hours [10 11]. It seems, therefore, as might have been expected, that in soil many anaerobic processes take place much more slowly than aerobic processes. Furthermore, since the decomposition of amino acids is accompanied by the formation of large quantities of volatile fatty acid and by the liberation of about 80 per cent of their nitrogen as ammonia, there can be little cell synthesis during amino-acid decomposition. Under aerobic conditions, the decomposition of amino acids would be expected to leave an appreciable residue of cellular material in the soil, material that would assist the binding of soil particles and so improve soil structure. The volatile fatty acids produced from amino acids under anaerobic conditions would on the other hand be expected to have little effect on soil structure apart from that resulting from leaching of calcium, sodium, *etc.*

It is hoped in future experiments to determine the characteristics

of soil metabolism in the region of transition from aerobic to anaerobic amino acid metabolism in different soils.

SUMMARY

1. Anaerobic amino-acid breakdown in a garden soil was accompanied by the formation of large quantities of volatile fatty acid and ammonia (plus indole in the case of tryptophan).

2. Hydroxyproline, leucine, methionine, phenylalanine, proline, threonine, tryptophan, tyrosine, and valine were found to be "resistant" and decomposed more slowly than did alanine, arginine, aspartic acid, cystine, glutamic acid, glycine, histidine lysine, and serine. Casein hydrolysate was rapidly and completely deaminated.

3. The slow rates of breakdown of the "resistant" acids when tested singly could not be attributed either to the effects of adsorption on the soil surface or to elimination of an essential Stickland reaction.

4. The effect of glucose and of nitrate on anaerobic amino-acid metabolism have been tested. With the exception of DL-methionine nitrate acted as an hydrogen acceptor in the anaerobic deamination of the "resistant" acids, glucose had no effect on their anaerobic metabolism.

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REFERENCES

1 Bremner, J. M., Recent work on soil organic matter. J. Soil Sci. **5**, 214–229 (1954).

2 Bremner, J. M. and Shaw, K., Determination of ammonia and nitrate in soil. J. Agr. Sci. **46**, 320–328 (1955).

3 Bremner, J. M. and Shaw, K., Denitrification in soil. 1. Methods of investigation. J. Agr. Sci. **51**, 22–39 (1958).

4 Bremner, J. M. and Shaw, K., Denitrification in soil. 2. Factors affecting denitrification. J. Agr. Sci. **51**, 40–52 (1958).

5 Broadbent, F. E. and Stojanovic, B. F., The effect of partial pressure of oxygen on some soil nitrogen transformations. Soil Sci. Soc. Am. Proc. **16**, 359–363 (1952).

6 Conway, E. J., Microdiffusion Analysis and Volumetric Error (3rd Edn.). Crosby Lockwood & Son Ltd., London (1950).

7 Conway, E. J. and O'Malley, E., Microdiffusion methods. Ammonia and urea using buffered absorbents. Revised methods for ranges greater than 10 μg N. Biochem. J. **36**, 655–661 (1942).

8 Geisking, J. E., The clay minerals in soils. Advances in Agron. **1**, 159–204 (1949).

9 Goring, C. A. I. and Bartholomew, W. V., Microbial products and soil organic

matter. 2. The effect of clay on the decomposition and separation of the phosphorus compounds in micro-organisms. Soil Sci. Soc. Am. Proc. **14**, 152–156 (1949).

10 Greenwood, D. J., Studies on the breakdown of amino acids and other organic compounds in soil. Ph. D. Thesis, University of Aberdeen. (1957).

11 Greenwood, D. J. and Lees, H., Studies on the decomposition of amino acids in soils. I. A preliminary survey of techniques. Plant and Soil **7**, 253–268 (1956).

12 Happold, F. C. and Hoyle, L., The quantitative determination of indole in bacterial cultures. Biochem. J. **28**, 1171–1173 (1934).

13 Hawk, P. B., Oser, B. L., and Summerson, W. H., Practical Physiological Chemistry (12th Edn.), p. 109. J. & A. Churchill Ltd., London (1949).

14 Lees, H. and Quastel, J. H., Biochemistry of nitrification in soil. 1. Kinetics of, and the effects of poisons on, soil nitrification, as studied by a soil perfusion technique. Biochem. J. **40**, 803–815 (1946).

15 Lewis, D., Amino acid metabolism in the rumen of the sheep. Brit. J. Nutrition **9**, 215–230 (1955).

16 Lynch, D. L. and Cotnoir, L. J., The influence of clay minerals on the breakdown of certain organic substrates. Soil Sci. Soc. Am. Proc. **20**, 367–370 (1956).

17 Markham, R., A steam distillation apparatus suitable for micro-Kjeldahl analyses. Biochem. J. **36**, 790–791 (1942).

18 McLaren, A. D., The adsorption and reactions of enzymes and proteins on Kaolinite. 2. The action of chymotrypsin on lysozyme. Soil Sci. Soc. Am. Proc. **18**, 170–174 (1954).

19 Medical Research Council, A System of Bacteriology in Relation to Medicine **9**, p. 97. H.M. Stationery Office, London (1931).

20 Mortland, M. M. and Geisking, J. E., The influence of clay minerals on the enzymatic hydrolysis of organic phosphorus compounds. Soil Sci. Am. Proc. **16**, 10–13, (1952).

21 Nisman, B. S. The Stickland reaction. Bacteriol. Rev. **18**, 16–39 (1954).

22 Pinck, L. A., Dyal, R. S., and Allison, F. E., Protein-montmorillonite complexes, their preparation and the effects of soil micro-organisms on their decomposition. Soil Sci. **78**, 109–118 (1954).

23 Putnam, H. D. and Schmidt, E. L., Studies on the free amino acid fraction of soils. Soil Sci. **87**, 22–27 (1959).

24 Woiwood, A. J., A method for the estimation of micro amounts of amino nitrogen and its application to paper partition chromatography. Biochem. J. **45**, 412–417 (1949).

25 Walkley, A. and Black, I. A., An examination of the Degtjareff method for determining soil organic matter, and a proposed modification of the chromic acid titration method. Soil Sci. **37**, 29–38 (1934).

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NITRIFICATION IN SOILS AFTER DIFFERENT PERIODS OF DRYNESS

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It has been shown (Birch [1]) that the longer a soil remains in an air-dry state the greater are the amounts of carbon and nitrogen mineralised on moistening it. These amounts were found to be a significant linear function of the log. of the time the soil was in a dry state prior to moistening. Even short periods in the dry state were sufficient to produce considerably greater amounts of mineral nitrogen, on moistening, than soils that had not been given a preliminary drying. Harpstead and Brage [8] have also reported recently that drying and storage of soils leads to a pronounced increase in their nitrifying ability, a result which they ascribe to changes in the relative numbers of various micro-organisms in the soil.

The experiment from which the foregoing results were quoted is now complete and is described below. It shows the effect of different periods in the dry state on subsequent nitrification in soils with carbon contents ranging from 1 to 7 per cent. The range of soils and drying periods covered are, it is hoped, sufficiently wide for the results to serve as a general guide in soil-nitrogen studies, particularly in countries with pronounced wet and dry seasons. The application of the results is discussed in some detail, particularly with respect to soil nitrogen. Brief reference is also made to carbon rundown in soils.

EXPERIMENTAL

Each sample was made up by mixing together several samples of about the same carbon content. Thus a number of soils containing about 1 per cent carbon were mixed, and similarly with the others. No attempt was made to

select the soils according to types. The soils had been in an air-dry state in the store for over a year. Table 1 shows some of their characteristics.

TABLE 1

Characteristics of soils				
% C (1)	% C (2)	% N	C/N	pH
6·90	9·18	0·663	13·8	5·48
6·28	8·35	0·693	12·1	6·13
5·48	7·23	0·593	12·3	5·92
4·22	5·61	0·460	12·2	6·22
3·14	4·18	0·330	12·7	6·16
2·06	2·73	0·210	13·0	5·82
1·00	1·33	0·095	14·0	6·05

C(1) Walkley-Black [14] (uncorrected value).

C(2) Walkley-Black value × 1·33, from which the C/N ratios are derived.

The range of carbon contents was based on the uncorrected Walkley-Black values because the organic material not oxidised by hot chromic acid (for which the correction factor 1·33 is made) is unlikely to be susceptible to microbial decomposition. The uncorrected values should, therefore, be a truer reflection of the material involved in nitrification and decomposition.

Decomposition and nitrification were carried out at approximately field capacity, the optimum moisture condition for such processes (Birch [2]). This was determined for each sample by soaking 20 g with water on a Buchner funnel, removing the excess with suction (using a water pump) and weighing.

To determine the effect of air-drying for different periods on subsequent nitrification the following procedure was adopted. Sufficient of the original air-dry composite sample was taken to give 300 g of soil at field capacity. Five 60-g portions of this moist soil were then put into separate respirometer vessels (8-oz. sample bottles) and left in the respirometer (Birch and Friend [3]) for 14 days. After this period the flush of decomposition, that characteristically follows the moistening of a dry soil, was over. The five portions were then recombined, water was added (if necessary) to bring the weight to 300 g, and the portions were treated as follows:

(1) 37-g were extracted for the determination of ammonium and nitrate nitrogen (see below), and 5-g were dried at 100°C for moisture content.
(2) 37-g were returned immediately to the respirometer.
(3) Five 37-g portions were spread out to dry for 3, 6, 9, 12 and 15 weeks with a few unavoidable exceptions noted in Table 2.
(4) Five 5-g portions were air dried as for (3).

Measurement (1) gives the amount of mineral nitrogen present at the start of the experiment proper and has to be subtracted from subsequent determinations to determine the effect of drying and no-drying on nitrification.

The 37-g portion returned immediately to the respirometer, treatment (2), was left there for 19 days, after which the amount of carbon mineralised

and the amount of mineral nitrogen present was determined as described below. The amount of nitrogen mineralised during treatment (2) is obtained by subtracting the amount present at the start of the treatment (measurement (1) above) from the amount present at the end of treatment (2). This represents the amount of nitrogen mineralised in a soil without a preliminary drying (since the drying effect following the initial moistening of the soil is eliminated by subtracting measurement (1)). This amount is strictly comparable with the magnitudes of nitrification following the moistening of the samples treated as in (3) which were dried at the same stage that the moist sample was returned to the respirometer (treatment (2)). The amount of carbon mineralised is, in all instances, given directly by the amount of carbon dioxide evolved during the 19-day decomposition period in the respirometer.

The five 37-g portions dried for different periods were treated as follows. After the required period of drying the sample was weighed and water added to bring it to 37-g. The moist sample was then kept in the respirometer for 19 days after which the amount of carbon mineralised and the amount of mineral nitrogen were determined. The latter, minus the amount present prior to incubation (measurement (1)) gives the amount of mineral nitrogen produced following the moistening of a dry soil. To determine the extra amounts of mineral nitrogen produced as a result of drying (followed by moistening) compared with no drying the values in column 4, Table 2, are subtracted from the values in columns 5, 6, 7, 8 or 9.

At the same time that the air-dried samples (treatment (3)) were moistened and put in the respirometer the original 5-g samples (treatment (4)) air-dried for the same period, were dried at 100°C for moisture content. The percentages moisture in the air-dry samples at the time of moistening are shown in Table 2. The carbon and nitrogen mineralisation data in this table are expressed as mg/100 g soil on an oven-dry basis. The moisture figures refer to the amount of water lost from the air-dry soils when kept at 100°C for 24 hours.

Determination of carbon and nitrogen mineralisation

Carbon. After the 19-day decomposition period in the respirometer the caustic soda solution (in which the evolved carbon dioxide had been absorbed) was transferred to a 100-ml volumetric flask and diluted immediately to the mark. A 5-ml aliquot was then titrated with $N/10$ hydrochloric acid using methyl orange (reading a). A second 5-ml aliquot was treated with excess 0·4 N barium chloride solution and again titrated, but against phenolphthalein (reading b).

$$(a - b) \times 22{\cdot}4 = \text{ml } CO_2 \text{ evolved.}$$

$$(a - b) \times 12 \quad = \text{mg C mineralised.}$$

Nitrogen. After the 19-day decomposition period the sample was mechanically shaken for 0·5 h with 50 ml $2N$ potassium chloride solution, 20 ml N hydrochloric acid and 30 ml water, and then filtered. A 50-ml

TABLE 2

Effect of different periods of dryness on amount of carbon mineralised and mineral nitrogen present after 19 days in the respirometer

1	2	3	4	5	6	7	8	9
Soil % C	Determination of	14 days after initial moistening	After a further 19 days	After 19 days moist preceded by air-drying for				
				3 weeks	6 weeks	9 weeks	12 weeks	15 weeks
6·90	C mineralised } mg/	129·4	58·3	92·0	121·6	137·7	146·3	161·6
	Mineral N } 100 g	25·49	27·35	40·55	47·66	50·90	53·66	55·44
	C/N		31·3	6·1	5·5	5·4	5·2	5·4
	% H_2O prior to moistening			7.57	9.72	5.71	4.17	5.71
6·28	C as above	124·6	57·3	79·1	83·7	98·4	97·7	101·1
	N ,, ,,	18·08	22·13	29·81	30·49	34·82	35·43	37·82
	C/N ,, ,,		14·4	6·7	6·7	5·9	5·6	5·1
	% H_2O ,, ,,			9·46	8·11	8·22	9·33	8·11
5·48	C ,, ,,	98·8	55·9	72·9	86·4	90·2	91·6	98·5
	N ,, ,,	13·82	18·46	24·40	26·14	25·87	28·86	29·56
	C/N ,, ,,		12·1	6.9	7·0	7·5	6·1	6·3
	% H_2O ,, ,,			5·19	8·86	5·26	4·00	3·95
4·22	C ,, ,,	64·3	34·0	—	47·3	60·6	61·3	64·5
	N ,, ,,	11·94	16·60	20·80	21·09	22·50	24·29	23·53
	C/N ,, ,,		7·3	—	5·2	5·7	5·0	5·6
	% H_2O ,, ,,			6·25	6·10	6·25	6·25	—
3·14	C ,, ,,	64·7	42·4	44·8	56·4	59·0	67·2	69·4
	N ,, ,,	11·39	14·57	16·42	18·31	19·60	20·19	19·60
	C/N ,, ,,		13·3	8·9	8·2	7·2	7·6	8·5
	% H_2O ,, ,,			6·25	6·41	—	8·75	5·12
2·06	C ,, ,,	55·2	—	35·3	52·2	65·7	71·6	69·8
	N ,, ,,	10·93	12·32	15·32	15·79	17·20	17·99	20·04
	C/N ,, ,,		—	8·0	10·7	10·5	10.1	7·7
	% H_2O ,, ,,			3·00	3·90	2·56	6·20	2·50
1·00	C ,, ,,	25·2	15·3	19·1	20·9	22·7	22·9	28·8
	N ,, ,,	5·80	7·54	8.97	8·65	10·95	9·86	10·64
	C/N ,, ,,		8·8	6·0	7·3	4·4	5·6	6·0
	% H_2O ,, ,,			0·46	1·13	0·00	1·14	1·14

aliquot of the filtrate was then steam distilled after adding 3-g of magnesium oxide. The ammonia liberated was collected in 10 ml of a 2% solution of boric acid containing methyl red-methylene blue indicator, and the amount determined by titration with $N/70$ hydrochloric acid. Devarda's alloy (2-g) was then added and the distillation repeated for the determination of nitrate nitrogen. The nitrogen mineralisation data therefore refer to both ammonia and nitrate nitrogen mineralised during decomposition, expressed however in units of nitrogen.

RESULTS

The amounts of carbon mineralised and the amounts of mineral nitrogen present after 19 days in the respirometer following the various treatments are given in Table 2. To obtain the amounts of nitrogen mineralised the mineral-nitrogen data in column 3 have to be subtracted from the other values in columns 4 to 9. The C/N ratios for the mineralisation process are calculated from these data.

Table 3 is a rearrangement of some of the data in Table 2 expressed in units of more practical application. It shows, as pounds of sulphate of ammonia per acre 6" (assuming this to weigh 2×10^6 lbs.) the amounts of mineral nitrogen produced during 19 days with no drying and after drying for different periods, followed by 19 days in the moist state. The extra amounts produced by drying, compared with no drying, are also shown in the same units and as a percentage of the no-drying treatments.

Table 3 should be of general application since it has been shown (Birch [2]) that the magnitude of decomposition on moistening a dry soil is largely a function, for a given drying period, of the carbon content of the soil and is little affected by the soil type with which the humus is associated, with the exception of peat soils and related soils.

The extra nitrogen and carbon mineralised after drying compared with no-drying, in relation to the period the soils were dry prior to moistening, is best expressed by a logarithmic relationship. The equations for each soil are given in Table 4, in which Y_n and Y_c are respectively the extra amounts of nitrogen and carbon mineralised (mg/100 g soil. Table 2, cols. 5, 6, 7, 8 or 9 minus col. 4) and X = log. of the number of weeks the soil was air dried prior to moistening.

TABLE 3

Nitrogen mineralisation data as pounds sulphate of ammonia per acre 6″, following the various treatments. See Table 2 for exceptions in the length of the drying periods

% C in Soil	During 19 days moist	Weeks air-dried prior to moistening					
		0	3	6	9	12	15
6·90	Amount mineralised. . . .	186	1,506	2,217	2,541	2,817	2,995
	Incr. over no drying. . . .		1,320	2,031	2,355	2,631	2,809
	% ,, ,, ,, ...		810	1,192	1,366	1,514	1,610
6·28	Amount mineralised. . . .	405	1,173	1,241	1,674	1,735	1,974
	Incr. over no drying. . . .		768	836	1,269	1,330	1,569
	% ,, ,, ,, ...		290	306	413	428	487
5·48	Amount mineralised. . . .	464	1,058	1,232	1,205	1,504	1,574
	Incr. over no drying. . . .		594	768	741	1,040	1,110
	% ,, ,, ,, ...		228	266	260	324	330
4·22	Amount mineralised. . . .	466	886	915	1,056	1,235	1,159
	Incr. over no drying. . . .		420	449	590	769	693
	% ,, ,, ,, ...		190	196	227	265	249
3·14	Amount mineralised. . . .	318	503	692	821	880	821
	Incr. over no drying. . . .		185	374	503	562	503
	% ,, ,, ,, ...		158	218	258	277	258
2·06	Amount mineralised. . . .	139	439	486	627	706	911
	Incr. over no drying. . . .		300	347	488	567	772
	% ,, ,, ,, ...		316	350	451	508	655
1·00	Amount mineralised. . . .	174	317	285	515	406	484
	Incr. over no drying. . . .		143	111	341	232	310
	% ,, ,, ,, ...		182	164	296	233	278

TABLE 4

Relationship between extra nitrogen and carbon mineralised and period the soils were dry prior to moistening

Soil	Extra N mineralised	Extra C mineralised
6·90% C	$Y_n = 20{\cdot}2X + 4{\cdot}2$ ($r^2 = 0{\cdot}997$)	$Y_c = 90{\cdot}9X - 8{\cdot}7$ ($r^2 = 0{\cdot}998$)
6·28% C	$Y_n = 11{\cdot}2X + 1{\cdot}5$ ($r^2 = 0{\cdot}901$)	$Y_c = 32{\cdot}1X + 5{\cdot}7$ ($r^2 = 0{\cdot}889$)
5·48% C	$Y_n = 6{\cdot}8X + 2{\cdot}3$ ($r^2 = 0{\cdot}775$)	$Y_c = 33{\cdot}2X + 2{\cdot}1$ ($r^2 = 0{\cdot}956$)
4·22% C	$Y_n = 4{\cdot}8X + 1{\cdot}2$ ($r^2 = 0{\cdot}786$)	$Y_c = 43{\cdot}7X - 19{\cdot}6$ ($r^2 = 0{\cdot}947$)
3·14% C	$Y_n = 5{\cdot}2X - 0{\cdot}40$($r^2 = 0{\cdot}899$)	$Y_c = 35{\cdot}0X - 14{\cdot}3$ ($r^2 = 0{\cdot}976$)
2·06% C	$Y_n = 6{\cdot}2X - 0{\cdot}59$($r^2 = 0{\cdot}837$)	—
1·00% C	$Y_n = 2{\cdot}6X - 0{\cdot}07$($r^2 = 0{\cdot}526$)	$Y_c = 11{\cdot}4X - 2{\cdot}54$($r^2 = 0{\cdot}743$)

The equations are of the form $y = ax + b$. Since $\log 1 = 0$, the b values in the above equations show the extra amounts of

nitrogen mineralised after 1 week's air-drying. Further, from the equations, no extra nitrogen is mineralised until the soils have been exposed to the drying process for longer than 0·62, 0·73, 0·46, 0·56, 1·20, 1·25, and 1·06 weeks for the 6·9 to 1·0% C soils respectively. Presumably these are the periods required for the moist soils to reach the air-dry state at which the drying effect first starts to manifest itself. Clearly the equations cannot hold for very long periods, but for the lengths of dry periods found in agriculture they are applicable.

DISCUSSION

The following discussion deals (a) with the results themselves, (b) with the application of these results, and (c) with the material and mechanism possibly involved.

(a) *The results*

Table 3 shows that for a given drying period the amounts of mineral nitrogen produced on moistening are directly related to the carbon contents of the soils with, however, outstandingly high values for the 6·90% C soil. Carbon mineralisation is also generally proportional to the carbon content of the soil (see Table 2), as already noted for other soils (Birch [2]). The relationships between carbon content of the soil and carbon or nitrogen mineralisation is less well defined for the non-dried soils (Table 2, col. 4; Table 3, col. 3). Possibly non-humified material is also present which decomposes not only during the flush of decomposition of the rather nitrogen-rich humus but also afterwards, leading to the uptake of some mineral nitrogen by the micro-organisms. Such behaviour could apply to both the 6·90 and 2·06% C soils (Table 3). Consequently, since the magnitudes of the drying effect as a percentage of no-drying are partly a function of the values given in Table 3, Col 3., large values are obtained with these two soils.

Soil drying enhances the magnitudes both of carbon and nitrogen mineralisation (Tables 2 to 4). The C/N ratios for the mineralisation processes after drying and moistening (Table 2, cols. 5 to 9) are, however less than after no drying (Table 2, col. 4). Hence it appears that the rate of nitrogen mineralisation after moistening the soil falls off more rapidly than that of carbon mineralisation. It has been

shown (Birch [1]) that following the moistening of a dry soil two decomposition processes occur namely (1) decomposition of soluble organic material without nitrification and (2) decomposition at the organic surface with nitrification, the rates of both processes rapidly declining. Presumably drying has a more transient effect on (2) than (1) leading to an increase in the C/N ratios for the mineralisation process as decomposition continues. Prolonged decomposition of organic residues not affected by the drying *i.e.* non-humic material, would further enhance this effect particularly if this also involved utilisation by the micro-organisms of mineral nitrogen produced earlier during the flush of decomposition.

The C/N ratios for the carbon and nitrogen mineralised after drying and moistening are quite low. The true values are probably even lower with reference to the actual material mineralised for, as mentioned above, part of the carbon mineralised is derived from soluble organic material that produces no mineral nitrogen during decomposition. Hence it appears that the material involved in nitrification is very nitrogenous.

From Table 3 it is seen that the magnitude of the drying effect on nitrogen mineralisation is considerable. Thus, with the 6·9% C soil drying for 9 weeks or more leads to the production of extra nitrogen on moistening (compared with no drying) equivalent to over a ton of sulphate of ammonia per acre 6″. Even after 3 weeks drying (a condition likely to obtain in temperate countries) considerable extra amounts are produced, while after 9 weeks drying, a state frequently achieved under tropical conditions, the extra amount produced even for the least organic soil was 341 lb. The magnitude of these effects for a given soil dried for a given period will gradually diminish with each successive drying and rewetting as the supply of material involved is depleted.

(b) *Application of the results*

It should be borne in mind that the flush of decomposition and nitrogen mineralisation that follows the moistening of a dry soil is repetitive with successive drying and rewetting treatments. This pattern of behaviour and the possible mechanism underlying it (see Birch [1]), which is discussed briefly later, can be usefully applied to several soil problems. Table 3 shows the magnitude of the drying effect and Table 4 indicates that it conforms more or

less to a basic principle. The effect of drying therefore follows a predictable course. Some of the applications of the drying effect have already been mentioned (Birch [2]), but not in the light of the magnitudes of the effects involved. This is now possible following the quantitative studies described in the experimental section.

(1) Nitrogen-fertiliser trials. In view of the large amounts of mineral nitrogen produced on moistening a dry soil, the results of such trials will have to be considered in relation (a) to the time of laying down the trial *i.e.* whether this was in time to catch the flush of nitrate production at the start of the rains or not, and (b) in relation to the length of the dry period prior to the rains.

With regard to (a), if planting is carried out once the flush of nitrification is over the crop will depend on the slow rate of nitrification that then occurs (Birch [2], and Table 3, col. 3), if the nitrate produced during the flush is leached out. With the more humic soils nitrate production under continued moist conditions may suffice for the crop. With less humic soils, however, the rate of supply under these conditions may be inadequate, in which cased the time of planting may have a critical effect on the results.

With regard to (b), if the trials are laid down at the start of the rains, the length of the dry period prior to these will be important particularly for the less humic soils as shown in Table 3.

(2) Yield-rainfall relationships. These usually ignore the length of the dry season in relation to rainfall, though this, as shown above, is important with regard to its effect on the subsequent magnitude of nitrification. Yields under adequate rainfall but after different periods of dryness may be very different. An interesting example is reported by Laycock [10]. He found a depressing effect on tea yield of dry-season rain. The effect of rain during a normally dry period will be to shorten the length of the dry period prior to the main rains when growth occurs. Hence the dry-season rains will tend to reduce the amount of mineral nitrogen then produced, with a concomitant effect on yield. Laycock [10] also found that the increment in crop per inch of rain during the early period of the rains was twice that during the later period. A result which could be due, partly at least, to the flush of nitrate production that follows the moistening of a dry soil. A dry spell

during the rains should also lead to a flush of nitrate production when these are resumed. Wood [15] (private communication) has reported that a dry spell during the growth of tobacco in Nyasaland was followed, when rain again occurred, by the uptake of large amounts of nitrogen by the leaf with adverse effects on its quality. This again demonstrates the important effect of drying on subsequent nitrate production.

Dry season rains will, however, only be detrimental to crops planted later at the start of the main rains if the nitrate produced during the dry season rain is lost through leaching.

(3) Irrigation. Table 3 shows that even a short period in the dry state is sufficient to produce considerable amounts of extra nitrogen on moistening the soil. Where irrigation is practised a short period of surface soil drying should be possible without damage to the crop since this obtains moisture from the subsoil. Remoistening should then have an effect equivalent to a nitrogen application. A period of drying should, if possible, also be introduced between the end of the rains and the start of irrigation, otherwise nitrification will continue at the low rate characteristic of constant moist conditions. In fact Laycock's observation reported above on crop yields in relation to early and late rain can be directly applied to irrigation practices. For any irrigation scheme it would seem advisable to establish a relationship between degrees and periods of drying, and the amounts of nitrogen subsequently mineralised on moistening as described earlier in this paper.

(4) Rundown of soil fertility. This, for nitrogen is a spasmodic process with little loss during the dry season, rapid mineralisation (possibly with leaching) at the start of the rains and a relatively slow rate of mineralisation as these continue. As the soil gradually becomes exhausted of the organic fraction from which the mineral nitrogen is derived a stage will be reached when the amount of mineral nitrogen produced after a short period of drying will be inadequate for crop needs while that produced after a longer period will still be adequate (see Table 3 for possible examples). Hence in countries with two pronounced wet and dry seasons, as in certain parts of Kenya, nitrogen deficiency will first be apparent after the shorter of the two dry periods. Glover and

Birch [4] have reported that a decline in soil fertility (as shown by declining maize yields over successive years) is beginning to show up in crops planted in the short rains (which are preceded by a short dry season) but not yet in crops planted in the long rains (which are preceded by a longer dry period), due account being taken of the differences in the amounts of rainfall.

(5) Effect of shade. The magnitude of nitrification following the moistening of a dry soil is a function not only of the period that the soil was dry prior to moistening but also of the temperature to which the soil was subjected (Birch [1 2]). Higher temperatures appear to accelerate the changes, induced by drying, responsible for the subsequent enhanced nitrification. Unpublished studies here have shown that the drying effect is somewhat enhanced at a temperature of 40°C and considerably enhanced at 60°C, the effect being almost equivalent to heating at 100°C. Since surface soils in the tropics may reach temperatures of 50 to 55°C the effect of shade in inhibiting the subsequent flush of decomposition on moistening should be considerable. Shade should also shorten the period, by delaying drying out, during which the soil is in the dry state. Hence shade should be conservative of organic matter and nitrogen, and it is interesting to note that Ap Griffith [5] found that shade considerably reduced the flush of nitrate when the soil was moistened.

(6) The available-nitrogen status of soils. From Table 3 it appears that any soil with 3 per cent C (approx. 6 per cent organic matter) or more should furnish adequate amounts of mineral nitrogen both from the initial flush of production on moistening (cols. 4 to 8) and from the slower rates of mineralisation that follow this (col. 3). These values refer to the amounts of nitrogen mineralised during 19 days. Soils with less than 3 per cent C produce comparatively little mineral nitrogen once the flush is over (see Table 3). Adequate amounts may or may not be produced during the flush of decomposition depending on the length of the dry period and the temperature reached. These soils appear then to be supplying considerably less mineral nitrogen once the flush is over, and as this stage of nitrification coincides with increased demands by the growing crop, nitrogen deficiencies may

occur. Another factor to be considered is that low-carbon soils are often of a sandy nature which means that nitrogen losses through leaching are likely to be considerable. With soils above 6 to 7 per cent C deviations in the behaviour described above are likely to occur as one moves from humic material of relatively low C/N ratio to undecomposed organic residues whose nitrogen content is such that decomposition to the humic state may be attended by uptake of mineral nitrogen by the micro-organisms. The general relationships described are therefore likely to be valid with soils containing up to 7 per cent C (Walkely-Black, uncorrected) but not above this level.

(7) Rundown of soil carbon. The amounts of carbon mineralised after drying and rewetting are shown in Tables 2 and 4. The magnitudes of these losses can best be appreciated when converted to other units. Thus the increased loss of about 20 mg C/100 g soil for the 6·28 and 5·48% C soils dried for 3 weeks is equivalent to 400 pounds C/acre 6″. To replace this about 3 tons of fresh organic matter would be required assuming that it contains 50 per cent water and 25 per cent C and that 75 per cent of the carbon is lost as carbon dioxide during its decomposition.

Following the decomposition of organic material (*e.g.* leaf or gelatine) added to the soil it has been found that the succeeding flushes of decomposition on drying and rewetting are bigger and more easily induced than prior to the decomposition of the added material. Hence the material involved in the drying effect appears to be a product of the decomposition of organic matter. Since this material is also decomposable it is difficult to build up organic matter in the soil. However, since its decomposition is controlled by the wetting and drying cycle its decomposition is spread over a number of seasons, which helps to explain the residual effects of added organic matter.

The flush of decomposition following each successive oven-drying and rewetting of a soil (Birch [2], Expt. 2) still occurs after 105 such treatments though the magnitude is now small. Each successive flush of decomposition decreases according to the equation:

$$Y_c = 35{\cdot}9 - 15 \log T \quad (r^2 = 0{\cdot}872,\ 103 \text{ observations})$$

where Y_c = mg C mineralised during 5 days after moistening the soil (35 g) and T = the number of treatments. $Y_c = 0$ when $\log T = 2{\cdot}393$, *i.e.* the flush of decomposition should no longer occur after about the 250th oven-drying and rewetting. It is estimated that about 73 per cent of the organic carbon originally present in the soil will then have been mineralised, leaving 27 per cent as microbially undecomposable material.

This experiment demonstrates the persistence of the decomposable organic fraction in the soil. Admittedly the soil had initially a high carbon and nitrogen content, but during these successive decompositions no additions were made to the soil (such as would occur under field conditions and the carbon dioxide evolved was adsorbed by sodium hydroxide solution and was therefore not available for synthesis of organic compounds by the micro-organisms.

Under the milder conditions of drying in the field the organic fraction might be expected to persist over a greater number of dryings and rewettings. Since the number of these will be a function of time the rundown of soil carbon under bare fallow and in countries with well-defined wet and dry seasons should, in the light of the foregoing equation, be a function of the log. of the time.

(c) *The material and mechanism involved*

As mentioned in the previous paragraph the material involved in the drying effect is the product of the decomposition of organic matter. The material could be microbial remains accumulated over a long period. The low C/N ratios for the mineralisation process (Birch [1]) indicate that it is very nitrogenous. Soil organic matter is chiefly colloidal and is more or less characterised by two fractions (a) a labile or nutritive fraction and (b) a residual fraction resistant to microbial action. It is evident therefore that only part of the humus is likely to be involved in the drying effect.

It seems probable then that the material involved is a nitrogenous colloid which, in the dry state is in the gel form (Haines [6]). With regard to the effect of ageing and drying Lykov [11] refers to gels that become porous on drying, while Vold *et al.* [13] found that as gels age the mesh structure disintegrates and the constituent particles agglomerate into coarse aggregates. A simple observation here showed that when a jelly made out of gelatine dried out in

a beaker a stage was reached when it split into flakes which broke away from the bottom of the beaker and curled up thereby practically doubling the surface area exposed. It is possible that similar behaviour occurs in the soil where organic colloids are present as thin layers. This observation demonstrates that drying could increase the surface area, and the effect would be of considerable magnitude where large surface areas, as in soils, are involved. An alternative possibility is that the porosity of the gels is increased by drying, as mentioned above and discussed in more detail elsewhere (Birch [1]). Either change would lead to increased surface area. The hypothesis previously advanced (Birch [1]) to explain the drying effect appears therefore to be feasible. This postulates that, on drying, the surface area of the organic gel is increased. On moistening it decreases, the process being reversible, as long as the gel persists in the soil. Immediately on moistening a dry soil a greater surface area is exposed to both solution and decomposition processes than later on after moistening when the reversible process has taken place. It has been shown (Birch [1]) that the organic material that goes into solution on moistening a soil decomposes rapidly, but without nitrification. The enhanced nitrification that also occurs when a soil is moistened must, therefore, involve decomposition, accompanied by nitrification at the gel surface, the rate of nitrification declining as the surface area decreases under moist conditions. In connection with surface decomposition of gels Khudyakov [9] reports that there are bacteria that are capable of breaking down organic-mineral gels, while Rosen and Sajewa [12] state that various organisms can penetrate agar and gelatine by virtue of a force similar to diffusion. Similar behaviour may occur in soils and the persistence of the nitrogenous organic fraction in spite of its inherent decomposibility (as shown by the rapid decomposition that occurs for a short period after each successive drying and rewetting) may be due to the fact that only for a short period after a dry soil is moistened is a relatively large area exposed to decomposition and nitrification processes.

In addition to the effect of drying mentioned above there is also the probability that enhanced nitrification on moistening also occurs (a) as a result of the high microbial activity associated with a freshly developing population and (b) as a result of the decom-

position of dead micro-organisms, killed during the preceding drying. Whatever the factor or factors involved it is evident that soil drying leads to the production of surprisingly large amounts of mineral nitrogen on moistening. Since the amount produced is a function of the humus content of the soil and the log. of the period the soil is in a dry state prior to moistening and since, after moistening, the decline in the rate of nitrification conforms approximately to a first-order reaction (Birch [1]. Calculation subsequently derived from Expt. 8) it is evident that nitrification in soils follows a more or less predictable course since it conforms to certain basic principles. The temperature effect remains to be studied and this could be important under tropical conditions where high surface temperatures are reached. Possibly the magnitudes of the drying effect determined under laboratory conditions (at about 25°C) give an under-estimate of the effect likely to obtain in the field. Hagenzieker [7] however found a topsoil flush of mineral nitrogen in a grey sand equivalent to 224 lb. sulphate of ammonia per acre and this seems to tally with the data for the low-humic soils in Table 3.

SUMMARY

An experiment is described in which soils with carbon contents ranging from about 1 to 7 per cent (Walkely-Black, uncorrected) were air dried for 3, 6, 9, 12, and 15 weeks and then remoistened. The amounts of carbon mineralised and ammonia and nitrate-nitrogen subsequently produced during 19 days were then determined and compared with the amounts mineralised from a non-dried soil. For a given drying period the amounts of carbon and nitrogen mineralised were proportional to the carbon content of the soil while, for a given soil, they were found to be a significant linear function of the log of the time the soil was in an air-dry state prior to moistening. With high-humic soils drying produced extra nitrogen on moistening sometimes equivalent to over 1 ton of sulphate of ammonia per acre. Even with low-humic soils values of about 300 pounds were common.

The results of the drying effect are tabulated both in conventional units and as pounds of sulphate of ammonia per acre 6". In the latter units the table should more readily serve as a general guide in applying the results. The application of the results to nitrogen fertiliser trials, irrigation, rundown of soil fertility and soil carbon, the effect of shade *etc.* is then discussed. The material and mechanism possibly involved is also considered. The results should be of fairly wide application since a basic principle operating on material (humus) of more or less uniform composition and common to all soils is involved.

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REFERENCES

1 Birch, H. F., Further observations on humus decomposition and nitrification. Plant and Soil **11**, 262–286 (1959).
2 Birch, H. F., The effect of soil drying on humus decomposition and nitrogen availability. Plant and Soil **10**, 9 (1958).
3 Birch, H. F. and Friend, M. T., Humus decomposition in E. African soils. Nature **178**, 500 (1956).
4 Glover, J. and Birch, W., Private communication.
5 Ap Griffith, G., Factors influencing nitrate accumulation in Uganda soil. Empire J. Exptl. Agr. **19**, 1 (1951).
6 Haines, W. B., The volume-changes associated with variations of water content in soil. J. Agr. Sci. **13**, 296 (1923).
7 Hagenzieker, F., Soil-nitrogen studies at Urambo, Tanganyika Territory, East Africa. Plant and Soil **9**, 97 (1957).
8 Harpstead, M. I. and Brage, B. L., Storage of soil samples and its effect upon the subsequent accumulation of nitrate nitrogen during controlled incubation. Soil Sci. Soc. Am. Proc. **22**, 326 (1958).
9 Khudyakov, Ya. P., The effect of micro-organisms on soil colloids. Pedology **7**, 48 (1943).
10 Laycock, D. H., An empirical relationship between rainfall and tea yields in Nyasaland. Paper presented at the Specialist Committee Meeting on Soil Fertility held at E.A.A.F.R.O. (February 1958).
11 Lykov, A. V., Theory of migration of moisture in capillaryporous colloidal bodies. Kolloid Zhur. **9**, 117 (1947).
12 Rosen, P. and Sajewa, S., The penetration of bacteria through solid colloid media. Z. Immunitätsforsch. **61**, 410 (1929).
13 Vold, R. D., Coffer, H. F., and Baker, R. F., Rheological studies and electron microscopy of calcium stearate-cetane gels. Inst. Spokesman Natl. Lubricating Grease Inst. **15**, 9 (1952).
14 Walkley, A. and Black, I. A., An examination of the Degtjareff method for determining soil organic matter, and a proposed modification of the chromic acid titration method. Soil Sci. **34**, 29 (1934).
15 Wood, R. A., Private communication.

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